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GENETICS

A PERIODICAL RECORD OF INVESTIGATIONS
BEARING ON HEREDITY AND
VARIATION

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GENETICS

A PERIODICAL RECORD OF INVESTIGATIONS BEARING ON HEREDITY AND VARIATION

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CORRIGENDA

Page 62, line 16, for "83" read "82."

Page 64, line 24, for "27" read "37."

Page 70, line 32, for "23.52" read "23.53."

Page 73, line 12, for "sa" read "as", line 12, for "inbotany" read "in botany."

Page 131, for "Plate 1" read "Plate 2."

Page 137, for "Plate 2" read "Plate 1."

Page 154, line 24, for " 'chromosome exchange' " read "interchromosomal crossing over or 'chromosome exchange'."

Page 156, last line, for " 'interchromosomal crossing over' " read "interchromosomal crossing over or 'chromosome exchange.' "

Page 160, last line, for " 'chromosome exchange' " read "interchromosomal crossing over or " 'chromosome exchange.' "

Page 216, line 5, after "old-gold," for the comma substitute a colon.
line 7, after "shown" use a comma instead of a period.
line, 8, for "The" read "the," and for "Ridgeway's" read "Ridgway's."
lines 9 and 11, for "strontiun" read "strontian."

Page 221, line 6 from bottom, for "five" read "six."

Page 225, first line of second paragraph, for "five" read "seven."

Page 230, line 11 from bottom, for "(1923)" read "(1922)."

Page 242, line 10, for "Percentage crossing over equals 110" read "Percentage crossing over equals 100."

Page 286, line 7, for "purple" read "not-purple."

Page 289, line 1, for "Median" read "Middle."
line 3, for "median" read "extra middle."
line 4, for "median" read "middle."

Page 290, lines 7-9, for "usually vertical- *vice versa*" read "dorso-ventral."

Page 331, second line of summary, for "with other" read "with certain other."

Page 317, line 4 from bottom, for "is always 7 so far as known" read "is 7."

Page 330, first line of last paragraph, for "proportion" read "proportions of,' and for "generation" read "generations."

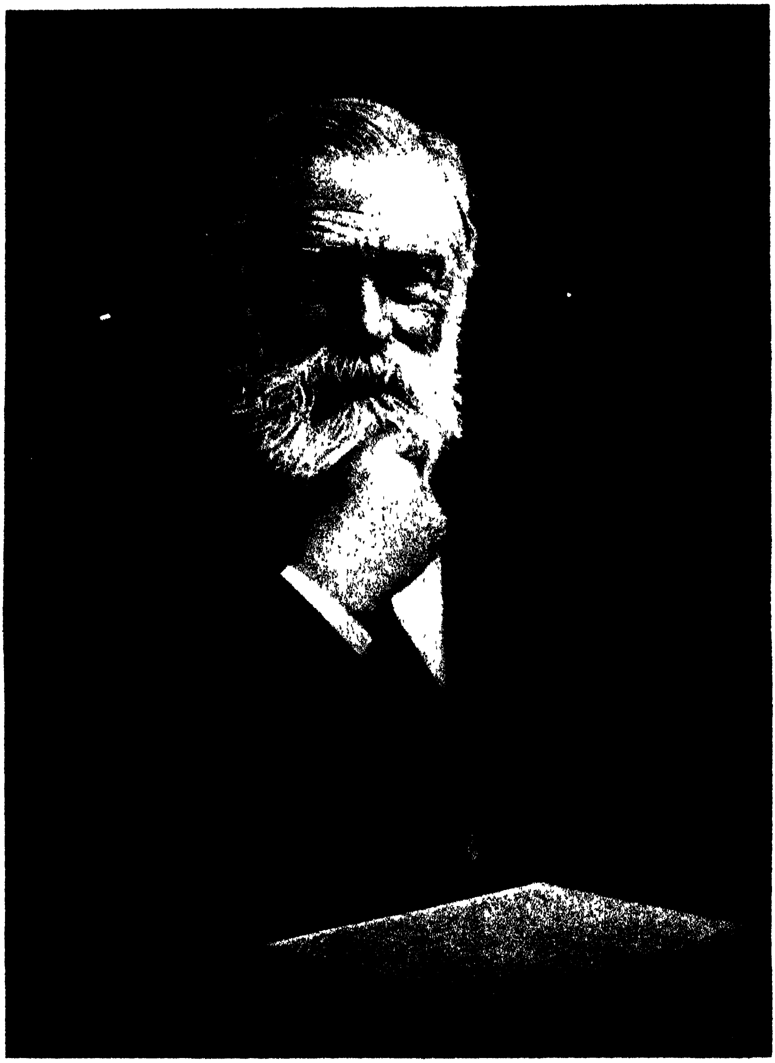
Page 356, line 6, omit comma after blue.

Page 358, line 10, "only" should be followed by a comma.

Page 368, line 3 from bottom, for "four genotypes, namely, BBLLee, BBLlee, BBllEE, and BBllEe," read "two genotypes, namely—BBLLee and BBLlee."

Page 423, for "Received March 1, 1925" read "Received March 1, 1926."

Page 502, Bibliography incomplete.



Hochachtungsvoll

Dr. W. O. Focke

WILHELM OLBERS FOCKE

(FRONTISPIECE)

Medizinalrat Doctor Wilhelm Olbers Focke was born April 5, 1834, in Bremen, Germany, where he died September 29, 1922. All of his life was spent in or near Bremen, except his school years, 1853 to 1858, in Bonn, Würzburg, Vienna and Berlin. He was a practicing physician in Oberneuland 1859–1861 and then in Bremen; Director of the Bremen Hospital, 1864–1867; Police Physician, 1871–1886; Prison Physician at Oslebshausen, 1874–1901; Member of the Board of Health of Bremen, 1886–1904; Business Manager and Consulting Physician to the Board of Health, 1901–1904; Directing Physician and Vice-President of St. Jurgen's Asylum, 1896 1897.

Although pure science was only his avocation, he accomplished an amazing amount of work, not only in botany and genetics, but he was also author of many papers on the geology, topography and climatology of Lower Saxony, and of biographical sketches of many of the distinguished scientific men of Bremen.

In 1864 he was one of the founders of the Scientific Society of Bremen, and was most active in his participation in its activities. He edited its proceedings until 1895 and for many years gave numerous brilliant and finished lectures before its sessions. Shortly after he retired from the editorship of the Proceedings he was elected an Honorary Member of the Society.

He accepted the views of Darwin before these had gained general recognition and many of his earlier papers, published mostly in "Kosmos" and the Proceedings of the Austrian Botanical Society, discussed species formation and other matters relating to organic evolution.

While Focke is best known to geneticists for his critical compilation, "Die Pflanzen-Mischlinge," published in 1881, his greatest contribution was undoubtedly the application of genetical methods to taxonomic problems in such difficult polymorphic genera as Rosa and Rubus. "Die Pflanzen-Mischlinge" has the honor of having been not only for several decades the most important source-book and starting-point for researches in hybridization, but, according to the account of de Vries, this book formed an important connecting-link between Mendel's classic paper on the garden pea and the "rediscovery" of Mendelian heredity in 1900, for de Vries found the reference to Mendel's paper in L. H. Bailey's "Plant Breeding" (published in 1895), where it had been cited from Focke's "Pflanzen-Mischlinge."

In this work Focke referred to Mendel's papers as "particularly instructive" but gave no indication as to the nature of their instructiveness, and, strangely enough, praised Godron's studies as of the greatest importance in showing that true-breeding forms can result from hybridization, a fact best established by the wonderfully clear analysis given by Mendel. To the end of his life Focke remained indifferent to the problems of analysis of hybrids on the basis of single characteristics. His interest was centered more on the significance of hybridization as a species-forming process and in relation to taxonomy.

He began his important studies on the taxonomy of Rubus early and published his "Synopsis Ruborum Germaniae" in 1877, and did not rest his extensive labors with this important and difficult genus until the completion of his "Species Ruborum Monographiae Generis Rubi Prodromus," published in three parts in Bibliotheca Botanica 1910–1914. The great value of this work rests on FOCKE's extensive utilization of progeny tests for constancy of differentiating characters, and numerous hybridizations to determine relationships. He was scientifically active almost to the end of his life, and as late as 1920 he published a brief paper on "Old disappearing and newly arising plant species."

The photograph here reproduced was taken in 1907 on the occasion of Doctor FOCKE's Doctorial Jubilee. We are indebted to Doctor H. DUNCKER, of the UNIVERSITY OF BREMEN, and, through his mediation, to the relatives of Doctor FOCKE for the loan of such portraits as are available, thus making possible a choice of the one which seemed best adapted to our use. Doctor G. TISCHLER of the UNIVERSITY OF KIEL kindly loaned a prized letter from which the signature has been reproduced.

THE VARIABILITY OF CROSSING OVER IN MAIZE*

L. J. STADLER

College of Agriculture, University of Missouri, Columbia, Missouri

Received May 9, 1925

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INTRODUCTION

This study is concerned chiefly with the extent and nature of the variability of crossing over in a family (full sibship) of *Zea mays*. It is part of a general study of variation in crossing over in maize, in which it is proposed to parallel the variousstu dies of this problem in Drosophila, as far as the nature of the material will permit, and also to investigate certain phases of the problem for which the plant is well suited and the fly is not.

Although the excellent conditions for the experimental study of linkage in Drosophila cannot be duplicated in maize, with its much longer reproductive cycle and much smaller known linkage groups, the plant offers quite favorable material for work of this kind in one linkage group of three endosperm characters. As far as the inheritance of endosperm characters is concerned, the single grain is the individual, and each well-developed ear comprises a progeny of several hundred. Thus it is possible, when using these characters, to handle the large numbers necessary in a statistical study. EAST (1913) has shown that "double fertilization" regularly occurs in maize, and that endosperm characters, therefore, may safely be used to indicate the genetic constitution of the zygote.

The three linked genes used in this study are *C c*, which in the presence of certain other genes affects aleurone color; S_h s_h, which affects the degree of shrinkage of the endosperm at maturity; and W_x w_x which affects the

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* The GALTON AND MENDEL MEMORIAL FUND has contributed toward the cost of setting the tables accompanying this article.

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chemical composition of the endosperm. Their linkage relations, which have been studied extensively by KEMPTON (1919), HUTCHISON (1922), and others, are indicated in figure 1.

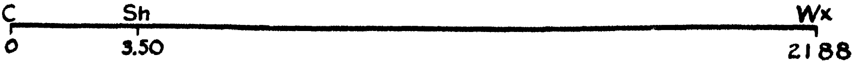

FIGURE 1.—Linkage relations of the factors C, S_h and W_x. (After HUTCHISON 1922).

The crossover percentages in these two sections of the chromosome are not ideal for the purpose, one being lower and the other somewhat higher than might be desired. The low crossover percentage is undesirable because the probable error computed by the usual formula,

$$E = .6745\sqrt{\frac{pq}{n}}$$

is unreliable when p is very small as compared with q, unless n is very large. The significance of differences in crossing over in this region is, therefore, somewhat doubtful when estimated by the use of this formula. The higher crossover percentage of the S_h-W_x region is undesirable because of the danger that undetected double crossovers may occur within the region. Since no locus between S_h and W_x is known at present, we cannot determine how frequently double crossing over may occur within this region. But double crossovers involving simultaneously the C-S_h and S_h-W_x regions are extremely rare, as HUTCHISON (1922) has shown and as the data of this paper confirm. It therefore seems probable that double crossing over within the S_h-W_x region is negligible as a source of error, although of course it is possible that double crossing over may occur freely in part of the C-W_x region while partly or wholly inhibited in another part.

The chief advantages of the material for the study of linkage problems are:

(1) Crossing over occurs freely in the development of both "male" and "female" gametes, and, since the plant is monoecious, both male and female gametes are produced by the same individual.

(2) A very large progeny may be produced from a single individual. The grains produced by a single heterozygous plant (which, backcrossed, indicate the crossover percentage among the female gametes) number one to two thousand in well-developed plants. The number of grains of multiple-recessive plants which may be pollinated from a single heterozygous plant (and which then indicate the crossover percentage among male gametes) is almost unlimited. By pollinating several ears it is possible to

produce a progeny of 5000 or more in each day's pollination throughout the pollen-shedding period, which, in well-developed plants, often lasts 20 days or more.

(3) Since pollination is easily effected and the sorting of the progenies may be done at one's convenience, it is practicable to determine the variability of crossing over in a large number of individuals of a single family grown simultaneously under uniform conditions.

(4) Tillers may be transplanted in early stages, and these under favorable conditions produce both seed and pollen. Separated parts of the same individual therefore may be exposed to different treatments to determine the effects of the treatments on crossing over.

(5) Samples of the young anthers can be taken at the time of the reduction divisions or even earlier, with no injury to the breeding powers of the plant. The stage in the development of the germ cells and the cytological effects of treatments may therefore be determined in the plants which are later to be used in breeding.

The series of studies of which this paper is a partial report was made possible through the generosity of Professor R. A EMERSON, who furnished me the foundation stock of this favorable material. I am indebted also to Professor EMERSON and to Professor E. M. EAST for criticism of the manuscript.

PREVIOUS STUDIES OF VARIATION IN CROSSING OVER

Crossing over has been found highly variable in Drosophila. GOWEN (1919), in an extensive biometrical study of crossing over in the third chromosome, found coefficients of variability ranging from 18 to 59 for the percentage of single crossovers in various regions, and from 67 to 110 for the percentage of double crossovers. In general the crossover percentages for the shorter regions were more variable than those for the longer regions. GOWEN concluded:

> "Crossing over is one of the most variable phenomena known, indicating that the mechanism behind crossing over is not as precise as that found in most physiological studies."

DETLEFSEN also found crossing over in Drosophila "an enormously variable phenomenon" as he remarks in reporting his experiments on selection for high and low crossing over in the sex chromosome (DETLEFSEN and CLEMENTE 1923).

The relation of specific environmental and genetic factors to crossing over in Drosophila has been studied by a number of investigators. A decrease in crossing over with age, limited to certain chromosome regions, was found by BRIDGES (1915). A striking increase in crossing over under extreme temperatures, either high or low, was found by Plough (1917, 1921), this effect being limited to the "susceptible" regions in which crossing over was affected by age. In Plough's experiments no significant difference in crossing over was found to result from varying amounts of moisture in the food, starvation, increased fermentation of food, or the presence of ferric chloride. Mavor (1923) and Mavor and Svenson (1924) found X rays effective in modifying the crossover percentage in both the second chromosome (susceptible to age and temperature effects) and the first chromosome (not susceptible to age and temperature effects). The same X-ray treatment increased the crossover percentage in the second chromosome and decreased it in the first. Plough (1924), in a preliminary trial of the effect of radium radiations, found a general tendency toward increased crossing over, especially after severe radiation.

Linkage variations due to germinal causes have been investigated especially by Sturtevant and Detlefsen. Sturtevant (1913, 1915, 1917, 1919) has reported several genes modifying the crossover percentage. The first gene of this kind reported, C_{III}, greatly decreases crossing over in the right-hand end of the third chromosome in flies which are heterozygous for it, though in flies homozygous for it crossing over in the same region is at least as high as normal. Similar results are given by C_{IIr} and C_{IIl}, which affect crossing over in the right-hand end and left-hand end, respectively, of the second chromosome, though the homozygous effect of C_{IIl} has not been determined. Two other crossover modifiers in the third chromosome have also been reported, C_{III_P} by Payne (1918) and C_{IIIM} by Bridges and Morgan (1923). These genes also reduce crossing over when heterozygous. Recently, Sturtevant (1921) has suggested the possibility that the reduction in crossover percentage ascribed to the action of C_{III} and C_{IIr} may be due rather to an inverted chromosome section, which might prevent synapsis when present in only one chromosome of a pair but not when present in both.

Detlefsen (1919), Detlefsen and Roberts (1920, 1921), and Detlefsen and Clemente (1924) have reported experiments in which the crossover percentage in the sex chromosome was greatly reduced by selection. Selection for increased crossing over was unsuccessful, a result attributed to increased double crossing over. A cross of "normal" stock with one of the low-crossover stocks produced by selection, gave results considered indicative of multiple-factor inheritance.

Gowen and Gowen (1922) have reported a third-chromosome gene which apparently inhibits crossing over completely in the female. They suggest that the results of Detlefsen's experiments may be accounted for by the action of one or more genes of this type.

Nabours (1919) reported that crossing over in Apotettix was apparently more frequent in the female than in the male. Castle (1919) also found that crossing over was more frequent in the female than in the male in rats. At the time, Castle doubted that this difference would be shown under uniform conditions, but in a later and more extensive study, Castle and Wachter (1924) found the higher crossover percentage in females to be consistent and to occur in mice as well as rats. No evidence of any relation of crossing over to age or seasonal conditions was found. Variability in crossing over in different male rats was found to be no greater than could be accounted for as the result of random sampling, with the possible exception of one individual.

Studies of variation in crossing over in plants have thus far been limited to comparisons of the crossover percentages in male and female gametes. Gowen (1919) pointed out that in the data given by Altenburg (1916) for linkage in Primula, the percentage of crossovers in the male gametes tested was significantly higher than that in the female gametes. Bateson (1921) stated that extensive data on linkage of magenta color and short style in Primula show closer linkage in the eggs than in the pollen, but that the linkage of green stigma and reddish stem is closer in the pollen than in the eggs. Eyster (1921) in a study of the linkage relations of sugary endosperm and tunicate ear in maize, found that the crossover percentage was 7.9 ± 2.0 higher among male than among female gametes. He also compared the C-W_x crossover determinations of Bregger (1918) in a similar way and found that in these trials the crossover percentage was 2.46 ± 1.11 percent higher in the male gametes.

In all of these trials one group of plants used as male parents was compared with another group used as female parents. Emerson and Hutchison (1921), pointing out that the results of such experiments may be due to environmental or genetic differences other than sex, submit data on crossing over in the production of male and female gametes in the same plants. Using the linkage of plant color and liguleless leaf in maize they found no significant difference in crossing over in the production of male and female gametes. In the linkage of colored aleurone and shrunken endosperm the percentage of crossovers was found to be slightly, but significantly, higher among the female gametes. They point out that this may be due to disturbing conditions such as temperature effects rather than to inherent difference in the mechanism of crossing over. Eyster (1922) has also supplemented his earlier study with a comparison of the crossover percentage in the S_u-T_u region in male and female gametes of the same plants, in which he found no significant difference between the sexes.

MATERIAL AND PROCEDURE

The family of plants used in this study (pedigree number 4003) was grown from seeds of a single ear. This ear was produced by pollinating a plant (1598-14) homozygous for colored aleurone, non-shrunken, starchy endosperm ($C\ C\ S_h\ S_h\ W_x\ W_x$) by a plant (1804-1) homozygous for colorless aleurone, shrunken, waxy endosperm ($c\ c\ s_h\ s_h\ w_x\ w_x$). Both parents were also homozygous for the genes AA, RR, and ii, the factors complementary to C in the production of aleurone color.

The two parental strains have been in genetic cultures for some years past. Family 1804, to which the male parent belonged, was grown from seed obtained from Doctor EMERSON in 1923. Family 1598, which included the female parent, was derived from a stock obtained in 1921 from Doctor E. W. LINDSTROM, who had grown it for several seasons. Through the kindness of Doctor EMERSON and Doctor LINDSTROM I have secured the pedigrees of these strains, which are shown in figure 2.

In order to favor maximum development of individual plants, the crop was planted one grain to a hill, 42 inches apart each way. The soil, which was naturally fairly fertile, was further improved by the application of a 4-12-3 fertilizer at the rate of 600 pounds per acre shortly before planting. Two rows of 50 hills each were planted to the heterozygous stock. About 75 of the 100 plants reached the flowering stage and all of these were used without selection.

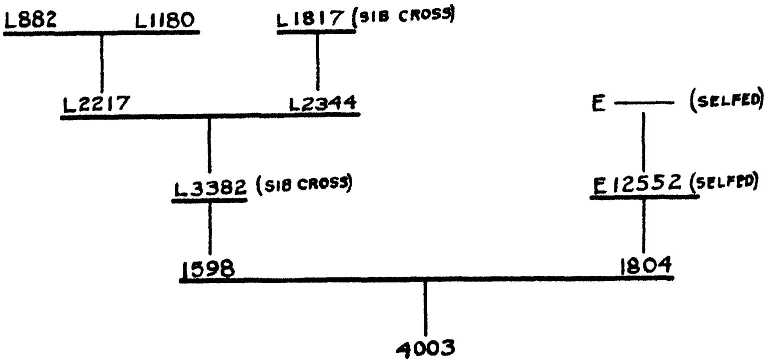

FIGURE 2.—Pedigree of family 4003. Pedigree numbers preceded by E refer to cultures of EMERSON, those preceded by L to those of LINDSTROM.

The crossover determinations were made by backcrossing with a multiple-recessive stock. The crossover percentage in male gametes was determined by pollinating at least one ear of the recessive stock from each tassel which shed pollen; in female gametes by pollinating two ears of the main stalk and the first tiller ear of all of the heterozygous plants which produced these ears. Of the heterozygous plants 58 produced 2 ears on the main stalk, and 31 produced a tiller ear. The multiple-recessive stock used was of low vigor and produced rather small ears. Some of these plants were so injured by chinch-bugs that they produced practically no grain. Consequently, the progenies representing the crossover series in the male gametes are smaller than those representing the female series, and for a few of the plants no determination of crossover frequency in male gametes could be made.

Pollen of the heterozygous plants was used regularly on the third day following the beginning of pollen shedding, to avoid error from any possible tendency towards change in crossing over with age of plant or inflorescence. These pollinations were made daily between 10 a.m. and noon. The ears of heterozygous plants were pollinated when the silks seemed well-enough developed to insure good filling of the ear.

In the sorting of the backcross progenies, each grain was individually tested for composition of endosperm (starchy or waxy) by a modification of the method suggested by WEATHERWAX (1923). Before shelling each ear a spot on each kernel was scraped to expose the endosperm, an operation quickly accomplished by the use of a small emery wheel. Before shelling and sorting the grains of each row, they were tested for endosperm composition by applying a touch of iodine solution to the scraped spot of each grain. The separations for aleurone color and shrinkage of the endosperm were clear and unmistakable except in a few ears of the heterozygous plants in which the shrinkage of the endosperm was very slight. These ears were separated only for C c and W_x w_x, S_h s_h being disregarded.

VARIABILITY OF CROSSOVER PERCENTAGE IN MALE GAMETES

The crossover percentage in male gametes, as shown by backcrossing to the multiple-recessive stock, was determined for 59 plants. The classified progenies of these plants are shown in table 14 (appendix). The extreme range in crossover percentage and the variability of crossing over in the C-S_h, S_h-W_x, and C-W_x[1] sections are shown in table 1 (page 8).

The variability of crossover percentages, as measured by the coefficient of variability, is decidedly higher in the shorter region than in the longer, as was found by GOWEN in Drosophila. In both the C-S_h and the S_h-W_x regions the variability is considerably less than that found by GOWEN in

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[1] The crossover percentage for the C-W_x region is determined by summation from those of the component regions (deducting twice the percentage of double crossovers). It is given to permit direct comparison with the plants in which shrunken endosperm could not be distinguished.

TABLE 1

Variability of crossover percentage in male gametes.

REGION	HIGHEST PERCENTAGE	LOWEST PERCENTAGE	MEAN PERCENTAGE	STANDARD DEVIATION	COEFFICIENT OF VARIABILITY
C-S_h	6.3	0.4	3.271±0.101	1.148±0.071	35.10±2.43
S_h-W_x	30.3	13.8	22.115±0.263	2.992±0.186	13.53±0.85
C-W_x	33.1	14.2	25.117±0.265	3.021±0.188	12.03±0.76

Drosophila in regions of similar length. For example, in the three sections of about 20 units length reported for Drosophila, the coefficients of variability were 31.0, 30.3, and 24.3, as compared with 13.5 in the S_h-W_x region of maize, and in the one region of about three units length reported for Drosophila the coefficient was 59.0, as compared with 35.1 for the C-S_h region in maize.

This lower variability of crossover percentages in the maize data does not necessarily mean that crossing over is a less variable process in maize than in the fruit fly, though of course this is a possibility. The two trials were under very different conditions and are not directly comparable. Two conditions in particular tend to make the variability lower in the maize data: (1) the plants compared are of the same parentage and are grown simultaneously under uniform conditions; and (2) the backcross progenies used in determining the crossover percentages are larger, so that the part of the variability which results from fluctuations of sampling is reduced.

The latter point may require some further explanation. Suppose we are dealing with a family of plants in which crossing over is perfectly uniform; that is, each plant *produces* exactly the same percentage of crossover gametes. Now in a study of the variability of crossing over in this family, we determine the crossover percentage in each plant from a backcross progeny of 400. The crossover percentages thus determined will not be uniform; they will fluctuate about the true value, with deviations dependent upon the size of the samples. For example, if the proportion of crossover gametes actually produced by each plant were 20 percent, the crossover percentage determined for each plant, from a backcross progeny of 400, would have a probable error of 1.33 crossover units. If crossover percentages were thus determined for 100 plants, about 50 of them would be below 18.67 or above 21.33 (20.00±1.33) and about 4 of them would be below 16 or above 24 ($20.00 \pm 3 \times 1.33$). Thus, there would be considerable variation in the crossover percentages determined, but it would be variation of sampling, not variation of crossing over. The series of 100 crossover values would have a mean of about 20 crossover units, a standard deviation of about 2 crossover units, and therefore a coefficient of variability of about 10 percent. If backcross progenies of only 100 instead of 400 had been used, the variability due to error of sampling, determined in the same way, would have been about 20 percent instead of 10 percent. If a chromosome region 10 units long, instead of 20, had been used, with backcross progenies of 100, the population of plants, though actually perfectly constant in crossing over, would have given a coefficient of variability of about 30 percent. Thus, when crossover frequency is measured by means of progenies of moderate size, one may find rather high apparent variability, though no lack of precision in the mechanism of crossing over is involved. This is true especially of the shorter chromosome sections, which explains, at least in part, the high variability found in the C-S_h section and in the short sections studied in Drosophila by GOWEN. Probably if large enough progenies were used in the measurement of crossover frequency in the short sections, their crossover percentages would be found no more variable than those of the long sections. But very large progenies would be required. In order to reduce to 10 percent the variability due purely to random sampling, progenies of more than 3000 each would be required for a section 3 units long, while progenies of 400 each would suffice for a section 30 units long.

The foregoing considerations suggest that crossing over may actually have been a very uniform process in the family of maize plants studied, and that such variations as were found may have resulted largely or wholly from error of sampling. Although the coefficients of variability determined are larger than would be expected to result from error of sampling alone, with samples of the size used, it is of interest to determine to what extent the variations may be accounted for by sampling fluctuations.

If all of the plants were actually identical in crossing over, (that is, if the variations found resulted wholly from error of sampling), only about half of the percentages of crossing over determined for individual plants should deviate from the mean percentage by as much as their probable errors, about 18 percent by as much as twice their probable errors, and so on, the expected frequencies being determined from tables of the probability integral. The actual frequency of such deviations, compared with the frequency expected if random sampling were the only cause of variability, is shown in table 2 on page 10.

It is clear that the variation between plants is wider than would be expected to result from error of sampling. In other words, the variability in crossover percentage found, though due in part to fluctuations of sampling, is due in part also to actual differences in the frequency of crossing over in different plants.

The correlation between crossover percentage in the C-S_h and S_h-W_x regions, in the male gametes of these 59 plants, was $-.247 \pm .082$. There was thus a slight but possibly significant tendency for a high crossover percentage in one section to be accompanied by a relatively low crossover percentage in the adjoining section.

TABLE 2

Variability of crossover percentage in male gametes compared with variability expected as result of error of sampling.

REGION	DEVIATION FROM MEAN, DIVIDED BY PROBABLE ERROR				
	1 or more	2 or more	3 or more	4 or more	5 or more
Expected	*29.50*	*10.46*	*2.54*	*0.41*	*0.04*
Observed:					
C-S_h	37	18	10	3	1
S_h-W_x	36	19	6	3	0
C-W_x	35	19	5	2	1

Variations in crossover percentage were not significantly correlated with height of plant, number of tillers, or number of ears. There was wide variation in vigor of growth among the plants of this family, plant height varying from 5 to 8½ feet, number of tillers from 0 to 4, and number of ears (including all which produced any grain whatever) from 1 to 8. But these differences had no discernable relation to the frequency of crossing over. There was a slight but possibly significant negative correlation of date of first pollen shedding and crossover percentage for the S_h-W_x region. The crossover percentage for C-S_h, which, as stated above, was negatively correlated with that for S_h-W_x, did not show any relation to date of first pollen. The correlation coefficients determined are shown in table 3.

TABLE 3

Correlation of crossover percentage in male gametes with certain plant characters.

PLANT CHARACTER	CORRELATION WITH CROSSOVER PERCENTAGE		
	C-S_h	S_h-W_x	C-W_x
Date of first pollen	$-.038 \pm .092$	$-.255 \pm .086$	$-.246 \pm .086$
Plant height	$.007 \pm .091$	$-.122 \pm .090$	$-.141 \pm .089$
Number of tillers	$.060 \pm .090$	$.134 \pm .089$	$.178 \pm .087$
Number of ears	$.022 \pm .090$	$.013 \pm .090$	$.036 \pm .090$

VARIABILITY OF CROSSOVER PERCENTAGE IN FEMALE GAMETES

A similar study was made of variation in crossover percentage in female gametes in this family. Crossover percentages in the tiller ears were found to differ significantly from those in ears of the main stalk, as will be shown presently. Since many of the plants did not produce tiller ears, it was necessary to disregard all tiller ears in determining the crossover percentages to be used in the study of variability. The crossover percentage in ears of the main stalk was determined in 70 plants. In 7 of these the shrunken character was indistinct, and was disregarded in making separations. There are available for the study of variation in crossover frequency in female gametes, therefore, crossover percentages in all three of the regions studied, in 63 plants, and in the C W_x region only in 7 additional plants. The classified progenies are shown in table 15 (appendix).

The variability of the crossover percentages thus determined is shown in table 4.

TABLE 4

Variability of crossover percentage in female gametes.

REGION	NUMBER OF PLANTS	HIGHEST PERCENTAGE	LOWEST PERCENTAGE	MEAN PERCENTAGE	STANDARD DEVIATION	COEFFICIENTS OF VARIABILITY
C-S_h	63	5.8	0.4	2.321±0.082	0.966±0.058	41.62±2.91
S_h-W_x	63	28.6	14.8	20.000±0.242	2.851±0.171	14.26±0.87
C-W_x	63	30.7	15.7	22.156±0.264	3.102±0.186	14.00±0.86
C-W_x	70	30.7	15.7	22.374±0.257	3.187±0.182	14.24±0.83

The variability is of the same order as that found in male gametes, though in all cases the coefficients of variability in the female gametes are slightly higher. This slight increase in variability might perhaps be accounted for by increase in sampling fluctuations accompanying the lower mean crossover percentages found in female gametes. On the other hand, sampling fluctuations should be reduced somewhat by the larger progenies used in determining crossover percentages in the female gametes.

The extent to which fluctuations of sampling may account for the variations observed is better shown by direct comparison of the observed deviations from the mean with the deviations expected from sampling errors alone. These are compared in table 5, which is prepared in the same way as table 2 for the male gametes.

Table 5

Variability of crossover percentage in female gametes compared with variability expected as result of error of sampling.

REGION	DEVIATION FROM MEAN, DIVIDED BY PROBABLE ERROR							
	1 or more	2 or more	3 or more	4 or more	5 or more	6 or more	7 or more	8 or more
Expected	*31.50*	*11.17*	*2.71*	*0.44*	*0.05*	*0.00+*	*0.00+*	*0.00+*
Observed								
C-S_h	44	29	16	6	3	0	0	0
S_h-W_x	45	32	16	9	4	1	1	1
C-W_x	43	32	20	12	5	2	1	1

The variability is well beyond that which may be accounted for by fluctuations of sampling. Obviously, the crossover percentage in female gametes, like that in male gametes, varied among individual plants within the population. The extent of variation in crossover percentage beyond that expected from sampling fluctuations is greater in the female gametes than in the male gametes.

The fact that there were individual differences between the plants of this family is further shown by the correlation between crossover percentages in different ears of the same plant. If crossover variations resulted only from error of sampling, different ears of the same plant should be no more similar than ears of different plants, for all ears would be simply random samples from the same population. In other words, if fluctuation of sampling were the only cause of differences between plants, the crossover percentages of different ears of the same plant would be uncorrelated variables. But actually the crossover percentages in different ears of the same plant were highly correlated. The 52 plants which produced two ears of the main stalk gave the following correlations for crossover percentage in the first and second ear:

Region	r
C-S_h	451 ± 078
S_h-W_x	.614 ± .061
C-W_x	.686 ± .048

These clearly significant correlations show that plants within this family differed significantly in crossing over, though they do not show whether such differences were due to germinal or environmental causes.

In the 63 plants in which crossover percentages for both C-S_h and S_h-W_x were determined, the correlation of crossover percentages in the two regions was .257 ± .081. This is contrary to the condition found in the male gametes, in which the crossover percentages in these two regions were negatively correlated to about the same extent.

The relation of crossover percentage in female gametes to plant height, number of tillers, number of ears, and date of first pollen shedding is shown in table 6.

TABLE 6

Correlation of crossover percentage in female gametes with certain plant characters.

PLANT CHARACTER	CORRELATION WITH CROSSOVER PERCENTAGE		
	C-S_h	S_h-W_x	C-W_x
Date of first pollen . .	.017 ± .088	.130 ± .086	.122 ± .087
Plant height......	−.153 ± 085	−.348 ± .077	−.340 ± .077
Number of tillers.. ..	−.094 ± .086	−.179 ± .084	−.180 ± .084
Number of ears.. .	− 193 ± .083	−.261 ± .080	−.296 ± .079

There is clearly a tendency toward negative correlation of crossover percentage and characters indicating vigor of growth, though all of the correlation coefficients are low. Plant height shows the closest correlation with crossover percentage, but number of ears and number of tillers also show some correlation, at least in the S_h-W_x and C-W_x regions. The correlation coefficients are lower for the C-S_h region, as was found also in the correlations determined above for crossover percentage in different ears of the same plant. The probable reason for this lower correlation in the C-S_h region is that the variations in this short region were more largely the result of sampling fluctuations than the variations in the longer regions. Date of first pollen was not significantly correlated with crossing over.

RELATIVE FREQUENCY OF CROSSOVERS IN MALE AND FEMALE GAMETES

The data already presented show a higher mean crossover percentage in male gametes than in female gametes. The data given for female gametes include several plants in which crossover frequency in male gametes was not determined, and those for male gametes include a few plants in which female gametes were not tested. Crossover percentages in both male and female gametes were determined in 54 plants, including 5 in which separations for S_h s_h could not be made in the ears representing the female gametes. These determinations are shown in table 16.

The low correlation between crossover percentages in the male and female gametes of the same plant is striking. The correlation coefficients are as follows:

Region	*Number of plants*	*Coefficient of correlation*
C-S_h	49	.212 ± .092
S_h-W_x	49	.075 ± .096
C-W_x	54	.122 ± .090

This is in agreement with the results of EMERSON and HUTCHISON (1921) on crossing over in the B-l_g and C-S_h regions in maize. These investigators found in plants extreme in crossover frequency in the gametes of one sex no marked tendency toward extreme results in the other sex. Correlation coefficients computed from the data given by them follow:

Region	*Number of plants*	*Coefficient of correlation*
B-l_g	19	.358 ± .135
C-S_h	20	.185 ± .146

The crossover percentages in male and female gametes of the same plants, computed from the totalled progenies of table 16, are shown in table 7.

TABLE 7

Crossover percentage in male and female gametes. Family 4003.

REGION	NUMBER OF PLANTS	MALE GAMETES TESTED		FEMALE GAMETES TESTED		DIFFERENCE
		Total progeny	Crossover percentage	Total progeny	Crossover percentage	Crossover percentage
C-S_h	49	23,348	3.42 ± 0.08	38,770	2.34 ± 0.05	1.08 ± 0.09
S_h-W_x	49	23,348	22.08 ± 0.19	38,770	19.99 ± 0.14	2.09 ± 0.24
C-W_x	54	24,982	25.19 ± 0.19	40,847	22.29 ± 0.14	2.90 ± 0.24

There is no question of the statistical significance of these differences, which are 8 to 12 times as large as their probable errors. The probability is inconceivably small that the total progenies representing male and female gametes are random samples from populations of gametes equal in crossover percentage. In both sections of the chromosome studied, the crossover percentage was distinctly higher in male gametes.

But we have seen that there are significant variations in crossing over between individual plants, and table 16 shows that there is great variation between plants, also, in relative frequency of crossovers in male and female gametes. It is possible that the difference in crossover percentage in the totalled data results from error of sampling the varying population of plants. In the method of comparison used above, the variation of plants is not a factor; the results are the same for any given crossover percentage and total number of grains, whether the plants making up the total are perfectly uniform or highly variable in crossing over.

The significance of the difference from this standpoint may be determined by averaging the differences in crossover percentage found in individual plants, and assigning to this average difference a probable error determined by BESSEL'S formula,

$$E = .6745\sqrt{\frac{\Sigma d^2}{n(n-1)}}$$

Computed in this way the differences are as follows:

Region	*Number of plants*	*Mean difference in crossover percentage*
C-S_h	49	0.935±0.115
S_h-W_x	49	2.065±0.397
C-W_x	54	2.607±0.375

Although the probable errors are considerably higher than in the preceding comparison, all three differences are distinctly significant. There is a high degree of probability, amounting to practical certainty, that an infinite number of plants of this population, tested under the same conditions, would have given a higher crossover percentage in male gametes than in female gametes, in both sections of the chromosome.

It does not follow, of course, that such a variation in crossing over is consistently associated with sex in maize. In fact, there are three good reasons for favoring the contrary view:

(1) Previously reported studies have given different results. Among the trials in which the same plants were used both as male and female parents, the only case in which a significant difference was found was one reported by EMERSON and HUTCHISON (1921), in which the crossover percentage in the C-S_h region was found significantly higher in *female* gametes. Other trials reported by EMERSON and HUTCHISON and by EYSTER (1922) showed no significant difference.

(2) Crossover variations analogous to those found in male and female gametes are found in different inflorescences of the same sex. For example, of the 21 plants of this family which produced both a main-stalk ear and a tiller ear, the crossover percentage (for C-W_x) was higher in the main-stalk ear in 17 cases. The mean of the differences in crossover percentage in the 21 plants was 2.26±0.40, which is of the same order as the differences reported above for male and female gametes. It is probable that these differences result from the different environmental conditions under which the gametes develop in the main stalk and in the tiller, which is always several days later in flowering.[2] Since distinct differences in

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[2] These data are presented and discussed in the next section of this paper.

crossover percentage are found in female gametes produced at different periods in the development of the plant, it is to be expected that crossover percentages may differ similarly in male and female gametes, for microsporogenesis and megasporogenesis also occur at different periods.

(3) The relative frequency of crossing over in the development of male and female gametes was found to differ consistently in different stocks. In addition to the data for family 4003, already reported, fairly extensive data are available in two other families, 4045 and 4070. Family 4045 was unrelated to family 4003, while family 4070 was related to both 4003 and 4045, its female parent being a full sib of that of 4003 and its male parent a full sib of that of 4045.

Two plantings of each of these families were made, the first on May 12 and the second on June 5. The later-planted series began to shed pollen about 10 days later than the earlier series. Thus the plants of the two series were likely to be under quite different weather conditions at any given stage of development. The crossover percentages for C-W_x, determined from the totalled progenies, are shown in table 8.

TABLE 8

Crossover percentage in male and female gametes. Families 4045 and 4070.

FAMILY	PLANTING	NUMBER OF PLANTS	MALE GAMETES TESTED		FEMALE GAMETES TESTED		DIFFERENCE CROSSOVER PERCENTAGE
			Total progeny	Crossover percentage	Total progeny	Crossover percentage	
4045	I	9	2745	25.36 ± 0.56	3797	24.73 ± 0.47	0.63 ± 0.73
4045	II	5	1999	29.01 ± 0.68	2045	27.38 ± 0.66	1.63 ± 0.95
4070	I	5	1668	26.26 ± 0.73	2177	21.18 ± 0.60	5.08 ± 0.94
4070	II	10	2992	28.24 ± 0.56	7284	24.71 ± 0.34	3.53 ± 0.65

Both plantings of 4045 gave an insignificant difference in crossover percentage in male and female gametes, while both plantings of 4070 gave a clearly significant difference in favor of the male gametes. Crossover percentages were higher in the later planting in both families, but increased in both male and female gametes, so that the relative frequency was not much affected.

VARIATION IN CROSSING OVER WITHIN THE INDIVIDUAL

To determine the extent to which crossing over in the development of male gametes might vary within the individual plant, successive pollinations were made from four plants at intervals of one or two days throughout the pollen-shedding season. The classified progenies of these plants

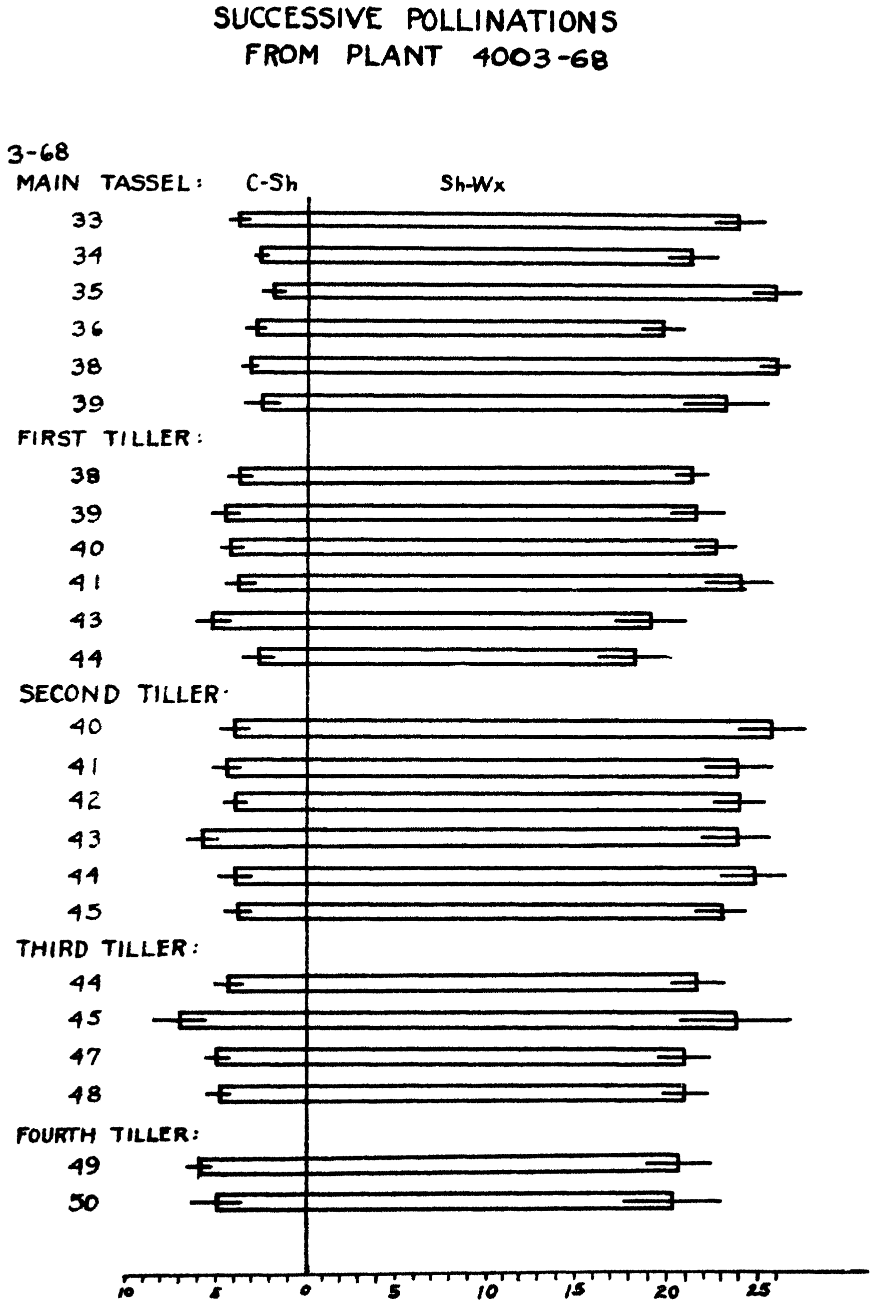

FIGURE 3.—Crossover percentages determined by repeated pollinations from plant 4003-68. Explanation in text.

are shown in tables 17 to 20, inclusive. The most extensive trial was made with plant 4003-68, from which pollinations were made daily, with two exceptions, through a period of 17 days. The plant produced 5 tassels, from each of which repeated pollinations were made. In all, 37 backcross ears were pollinated from this plant. The crossover percentages determined are shown graphically in figure 3. Similar but less extensive trials were made with plants 4013-2, 4017-5, and 4017-21. The results of these 3 trials are shown in figures 4 to 6, inclusive.

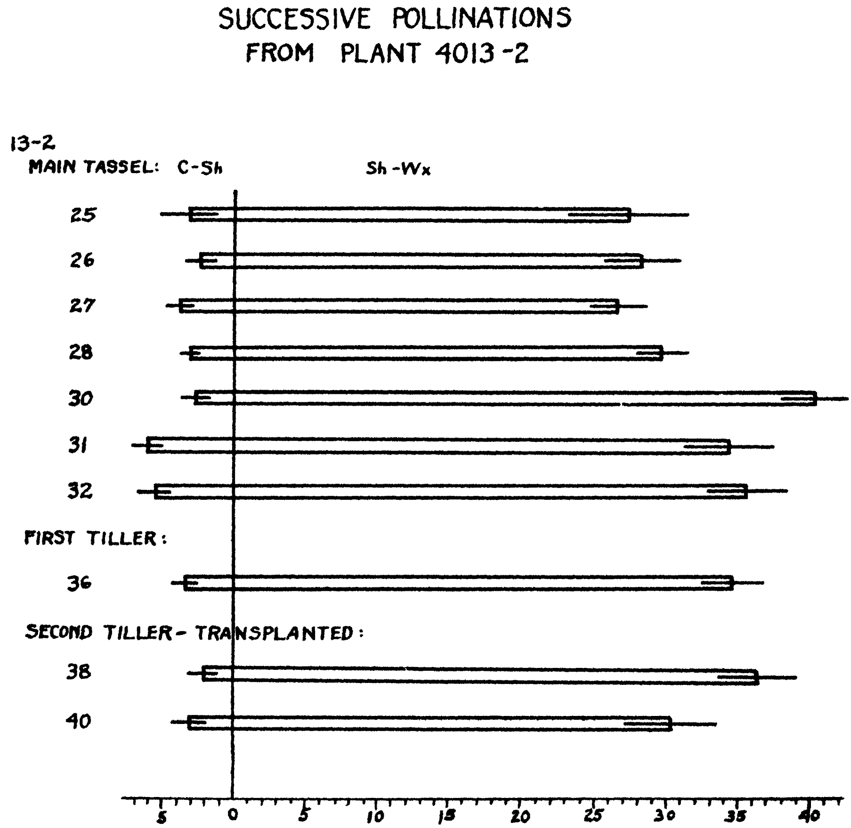

FIGURE 4.—Crossover percentages determined by repeated pollinations from plant 4013-2. Explanation in text.

In these graphs the crossover percentages for the C-S_h and S_h-W_x region are indicated by the length of the bars, while the probable error of each crossover determination is indicated by the length of the single line at the end of the bar. Thus, the first bar of figure 2 indicates crossover percentages of 3.7 ± 0.6 for C-S_h and 23.9 ± 1.4 for S_h-W_x. The dates given are continuous through July and August, July 31 being given as 31, August 1 as 32, etc.

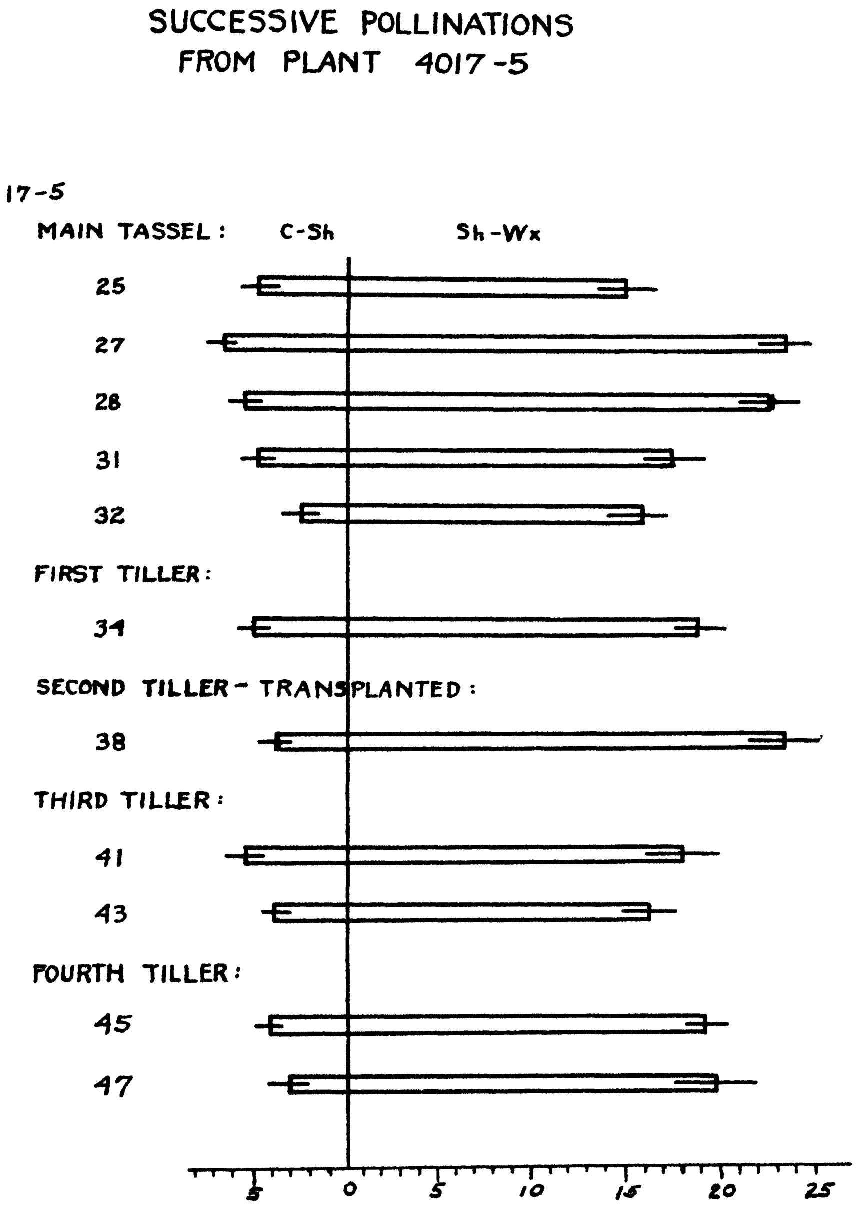

Figure 5.—Crossover percentages determined by repeated pollinations from plant 4017-5. Explanation in text.

The crossover percentages determined by successive pollinations from these 4 plants agree in indicating:

(1) There is no consistent increase or decrease in crossing over, with age;

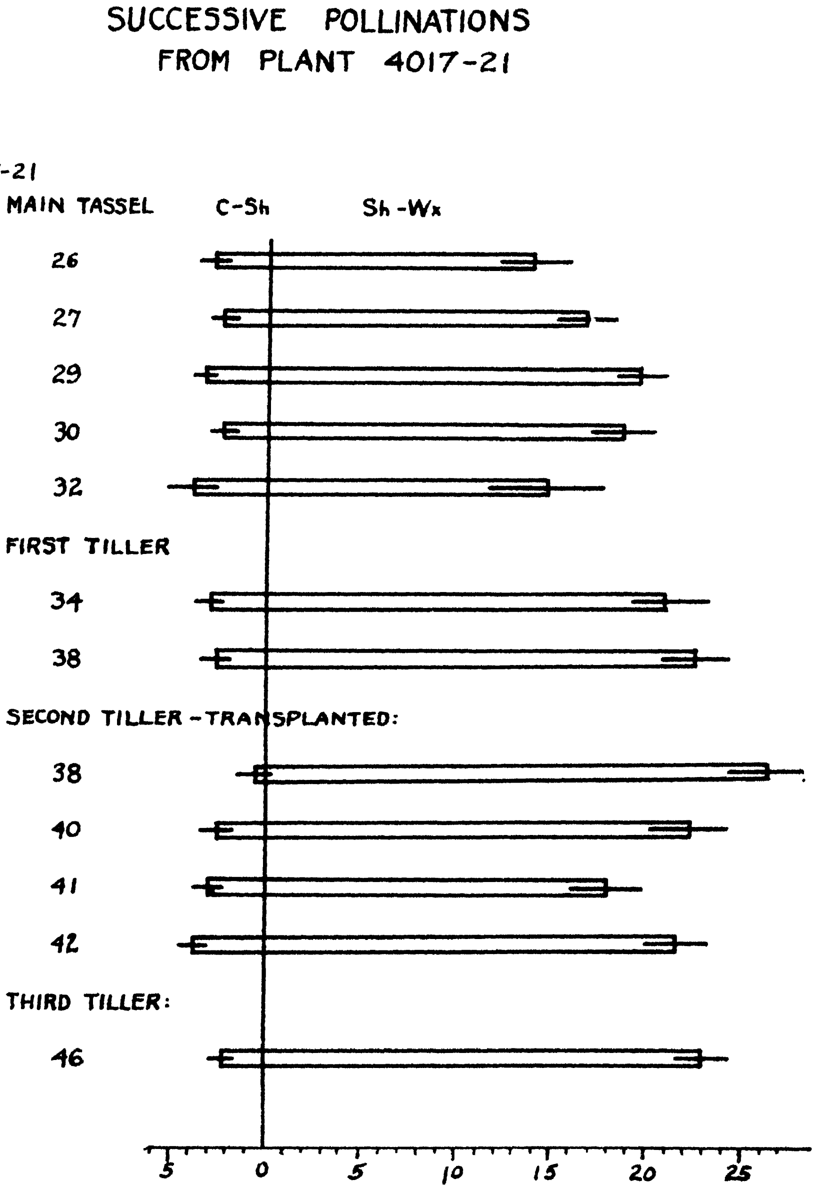

FIGURE 6.—Crossover percentages determined by repeated pollinations from plant 4017-21 Explanation in text.

(2) The fluctuations of the crossover percentage for the C-S_h region are not noticeably correlated, positively or negatively, with the fluctuations of the crossover percentage for the S_h-W_x region;

(3) The crossover percentages determined by pollinations from transplanted tillers were not significantly different from those of other tassels of the same plant; and

(4) The fluctuations in crossover percentage are surprisingly small when their probable errors are considered.

The possibility that these daily fluctuations in crossover percentage are due merely to error of sampling may be examined by the method which was used in studying the variation among different plants. In table 9 are shown the fluctuations from the mean, expected as a result of error of sampling, compared with the fluctuations observed. There are, as would be expected, considerable divergences from the expected proportions within individual plants, but in the aggregate, the agreement of expected and observed fluctuations is very close. In other words, crossover percentages determined by repeated pollinations from a single homogeneous supply of pollen would be expected to vary almost as widely

TABLE 9

Daily variation in crossing over compared with variation expected as result of error of sampling. Repeated pollinations from 3-68, 13-2, 17-5 and 17-21.

PLANT	NUMBER OF TRIALS	OCCURRENCE	DEVIATIONS FROM MEAN, DIVIDED BY PROBABLE ERROR					
			1.0 or more	1.5 or more	2.0 or more	2.5 or more	3.0 or more	3.5 or more
3-68	24	*Expected*	*12.0*	*7.5*	*4.3*	*2.2*	*1.0*	*0.4*
		Observed:						
		C-S_h	12	10	4	1	0	0
		S_h-W_x	11	6	4	1	1	1
13-2	10	*Expected*	*5.0*	*3.1*	*1.8*	*0.9*	*0.4*	*0.2*
		Observed.						
		C-S_h	4	2	1	0	0	0
		S_h-W_x	8	3	2	2	1	1
17-5	12	*Expected*	*6.0*	*3.7*	*2.1*	*1.1*	*0.5*	*0.2*
		Observed						
		C-S_h	6	4	3	2	2	1
		S_h-W_x	7	6	5	2	1	0
17-21	12	*Expected*	*6.0*	*3.7*	*2.1*	*1.1*	*0.5*	*0.2*
		Observed						
		C-S_h	4	3	2	1	0	0
		S_h-W_x	7	5	3	1	1	0
Total .	58	*Expected*	*58.0*	*36.2*	*20.6*	*10.7*	*5.0*	*2.1*
		Observed	59	39	24	10	6	3

as did the percentages determined by repeated pollinations throughout the pollen-shedding period.

Further evidence on the extent of variability within the individual plant is given by the crossover percentages determined by pollinations from main tassels and tiller tassels. In 19 plants of family 4003 crossover percentages were determined by pollination from both the main tassel and the first tiller tassel. These percentages are shown in table 10.

TABLE 10

Crossover percentage in main tassel and first tiller tassel. Family 4003.

PLANT NUMBER	FIRST TASSEL PERCENT	FIRST TILLER TASSEL PERCENT	DIFFERENCE PERCENT
13	19.4±2.1	30.4±2.2	11.0±3.1
16	26.9±1.7	30.6±1.8	3.7±2.5
18	23.6±1.4	26.0±1.7	2.4±2.2
23	27.0±2.0	26.7±1.5	−0.3±2.5
28	28.1±1.6	24.3±2.1	−3.8±2.6
41	29.5±1.8	26.1±2.2	−3.4±2.8
46	25.8±1.7	23.8±1.6	−2.0±2.3
54	24.8±1.4	25.7±2.2	0.9±2.6
55	27.4±1.7	21.9±1.9	−5.5±2.5
58	24.3±1.8	24.1±1.8	−0.2±2.5
61	25.3±1.9	26.3±1.7	1.0±2.5
65	27.3±2.5	29.7±1.6	2.4±3.0
66	19.8±1.9	22.0±1.5	2.2±2.4
68	27.5±1.4	25.0±1.3	−2.5±1.9
69	29.2±2.1	23.9±2.0	−5.3±2.9
73	25.2±2.4	21.8±2.4	−3.4±3.4
83	27.7±2.2	30.2±1.9	2.5±2.9
85	25.8±2.1	22.0±2.0	−3.8±2.9
99	29.1±1.5	26.1±1.7	−3.0±2.3

The tiller tassels do not differ consistently from the main tassels. In 8 cases the crossover value is higher in the main tassel; in 11 cases in the tiller tassel. Of the 19 differences, 11 are as great as their probable errors, 2 are twice as great, and 1 is three times as great,—again only slightly more variability than would be expected to result from error of sampling. The mean difference in crossover percentage is 0.37 ± 0.60, in favor of the main tassel. The difference is less than its probable error and of course has no significance.

Thus, in this material, crossing over in the production of male gametes has been a relatively constant character of the individual plant. Any environmental variation which has occurred has been too slight to be detected in populations of the size used in this study. Fortunately, the material used will permit a considerable increase in precision. By using large-eared recessive stocks and pollinating as many ears as possible from each sack of pollen taken from the heterozygous plant, it is possible to reduce the probable error of the crossover percentages representing each day's pollination, at least to 0.2 for the C-S_h region and 0.4 for the C-W_x or S_h-W_x regions. Fluctuations of 1 or 2 crossover units would then be clearly significant.

While crossing over in the production of male gametes varied little within the plant, it varied widely in different plants. The four plants from which daily pollinations were made are favorable material for this comparison, for their large total progenies establish their crossover percentages with a fairly high degree of precision, while the repeated pollinations demonstrate that daily fluctuations were a very minor factor in the differences. These four plants represent three families, of which two were very closely related. Family 4017 was produced by crossing two plants which were full sibs of the parents of family 4003. Family 4013 was not related to the other two families.

The crossover percentages determined from the totalled progenies of the four plants are shown in table 11.

TABLE 11

Individual differences in crossing over.

PLANT	MALE GAMETES			FEMALE GAMETES
	C-S_h	S_h-W_x	C-W_x	C-W_x
3-68	3.9±0.1	22.8±0.3	26.4±0.3	25.8±0.9
13-2	3.3±0.3	30.3±0.7	33.2±0.7	28.7±1.1
17-5	4.3±0.2	19.0±0.5	23.0±0.5	19.6±0.8
17-21	2.9±0.2	20.8±0.5	23.6±0.5	21.0±1.0

There are significant differences in crossing over between any two of the four plants compared. The most striking difference is between 4013-2 and 4017-5, which, as it happened, were planted on the same day, reached pollen-shedding on the same day, and grew under uniform conditions within 5 yards of one another. Plant 17-5, which had a much lower crossover percentage in the S_h-W'_x region, had a somewhat higher crossover percentage in the C-S_h region. The latter difference is perhaps insignificant, but there can be no doubt of the significance of the differences in the S_h-W_x region or in the C-W_x region taken as a whole, which are, respectively, 11.3±0.9 and 10.2±0.9. A similar difference in crossover percentage was found in the ears of these two plants, in which the difference in the crossover percentage for C-W_x was 9.1 ± 1.4. Unfortunately, separations for S_h s_h could not be made in the ears of plant 17-5, so crossover percentages in female gametes could not be determined for the C-S_h and S_h-W_x sections. For the C-W_x region, considered as a whole, crossing over was about 45 percent more frequent in plant 13-2, in the development of both male and female gametes.

In female gametes the crossover percentages in main stalks and tillers showed a relation very different from that found in male gametes. Of the 70 plants for which crossover percentages for C-W_x were determined, 21 produced a backcrossed ear on both the main stalk and the first tiller. The crossover percentages for these ears are shown in table 12.

TABLE 12

Crossover percentage in first ear and first tiller ear. Family 4003.

PLANT	FIRST EAR PERCENT	FIRST TILLER EAR PERCENT	DIFFERENCE PERCENT
7	24.3±1.3	27.0±1.3	2.7±1.8
11	22.6±1.3	26.2±1.4	3.6±1.9
13	20.5±1.5	22.5±1.6	2.0±2.2
16	19.8±1.4	20.9±1.2	1.1±1.8
17	20.5±1.3	24.1±1.3	3.6±1.8
19	20.5±1.6	17.7±2.2	−2.8±2.7
24	17.8±1.3	26.9±1.4	9.1±1.9
31	22.5±1.3	22.7±1.5	0.2±2.0
33	20.3±1.2	18.9±1.2	−1.4±1.7
34	23.1±1.2	25.0±2.1	1.9±2.4
46	25.5±1.3	26.6±1.3	1.1±1.8
52	22.2±1.3	22.0±1.3	−0.2±1.8
53	17.8±1.4	23.6±1.4	5.8±2.0
54	17.5±1.2	17.8±1.3	0.3±1.8
63	25.6±1.2	29.2±1.3	3.6±1.8
67	21.0±1.5	24.2±1.3	3.2±2.0
68	26.4±1.3	25.3±1.2	−1.1±1.8
69	22.9±1.2	25.7±1.4	2.8±1.8
78	27.6±2.3	28.8±1.4	1.2±2.7
85	17.3±1.5	22.4±1.6	5.1±2.2
99	20.0±1.3	25.7±1.3	5.7±1.8

The crossover percentages are consistently higher in the tiller ears. In only 4 of the 21 cases is the main-stalk ear higher in crossover percentage, and only one of these differences is as great as its probable error. The mean of the differences is 2.26 ± 0.40, or 5.65 times its probable error. Similarly, the crossover percentages determined from the totalled progenies are 21.26 ± 0.29 for the main-stalk ear and 23.96 ± 0.31 for the tiller ear, giving a difference in favor of the tiller ear of 2.70 ± 0.42. Thus, different ears of the same plant have undoubtedly differed significantly in crossover percentage.

Whether this difference is due to age or to different environmental conditions at a critical period cannot be certainly determined from the available evidence. Whatever the cause of the difference between inflorescences of main and tiller stalks, however, it is clear that crossing over was far more affected by it in female than in male gamete development.

The first and second ears of the main stalk were strikingly similar in crossover percentage, in the 52 plants which produced two main-stalk ears. In this family the two ears of the main stalk were usually of about the same size and silked on the same day. Presumably, they developed under practically the same environmental conditions. The differences between their crossover percentages were no greater than those to be expected from fluctuations of sampling, as shown in table 13.

TABLE 13

Differences in crossover percentage in first and second ear compared with differences expected from error of sampling.

	OF PLANTS	1.0 or more	1.0 or more	3.0 or more
Expected	*47*	*23.50*	*8.33*	*2.02*
Observed:				
C-S_h	47	23	8	0
S_h-W_x	47	20	7	0
Expected	*52*	*26.00*	*9.22*	*2.24*
Observed.				
C-W_x	52	25	6	1

SUMMARY

Among a maize population of full sibs grown under uniform conditions:

(1) Crossing over was found only moderately variable, when due allowance was made for fluctuations of sampling.

(2) Crossover percentages determined for the short C-S_h region were much more variable than those for the long S_h-W_x region. This is probably due to the fact that the former are much more greatly affected by errors of sampling.

(3) Crossover percentages in female gametes were more variable than crossover percentages in male gametes.

(4) Crossover percentages in the first and second ear of the main stalk were highly correlated.

(5) Crossover variations in male gametes were not correlated with plant height, number of ears, or number of tillers, but were possibly correlated, negatively, to a slight extent, with date of first pollen.

Crossover variations in female gametes were correlated negatively with plant height, number of ears, and possibly number of tillers, but were not correlated with date of first pollen.

(6) Crossover percentages in male and female gametes of the same plants were not significantly correlated.

(7) Crossover percentages were significantly higher in male gametes than in female gametes of the same plants.

Within the individual plant:

(8) Crossover percentages in male gametes, determined by repeated pollinations throughout the pollen-shedding season, did not differ significantly.

(9) Pollen of transplanted tillers did not differ significantly in crossover percentage from pollen of other tassels of the same plant.

(10) Crossover percentages in female gametes were significantly higher in tiller ears than in ears of the main stalk. In male gametes no such difference was found. Thus, crossing over in the development of female gametes was apparently affected by age or environmental conditions which had no distinct effect on crossing over in the development of male gametes.

Among plants of different families:

(11) A single random comparison of two plants from unrelated families showed a wide and clearly significant difference in crossing over. The differences were similar in male and female gametes.

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APPENDIX—Tables 14 to 22

TABLE 14

Crossover data, male gametes, family 4003.

PLANT NUMBER	NON-CROSSOVERS		CROSSOVERS IN REGION 1		CROSSOVERS IN REGION 2		CROSSOVERS IN REGION 1, 2		TOTAL
3	136	115	1	2	33	34	0	0	321
5	59	84	0	4	22	15	1	0	185
7	242	264	13	12	68	64	0	2	665
10	131	109	4	3	33	25	1	0	306
11	245	203	10	8	51	48	2	3	570
12	104	100	5	1	47	26	1	0	284
13	223	211	8	10	63	68	0	0	583
16	186	216	10	7	75	69	0	0	563
18	309	290	16	22	66	87	1	1	792
19	102	95	9	6	37	26	0	0	275
20	81	91	4	1	36	27	0	0	240
23	183	247	13	6	78	63	0	1	591
24	101	105	1	0	13	20	0	0	240
27	101	101	5	2	29	27	0	0	265
28	175	208	5	8	72	55	1	0	524
29	150	148	4	1	43	36	0	1	383
30	178	165	11	5	40	59	0	2	460
33	415	436	14	9	114	114	0	0	1102
34	73	53	3	4	15	18	0	0	166
35	122	114	7	6	32	32	0	1	314
40	92	82	6	3	19	15	0	0	217
41	164	157	9	6	49	62	0	0	447
44	62	74	1	3	26	27	0	0	193
46	209	259	18	14	68	53	1	0	622
47	74	77	2	2	29	25	0	0	209
52	279	296	8	9	91	88	1	1	773
53	121	108	4	4	37	41	0	0	315
54	232	250	10	4	81	66	0	0	643
55	307	291	18	19	76	81	0	1	793
58	209	194	5	11	51	62	0	1	533
60	171	170	16	13	43	44	0	0	457
61	285	301	9	12	83	99	0	1	790
63	78	100	7	2	21	25	0	0	233
65	156	178	8	8	62	58	0	0	470
66	250	228	14	12	54	48	0	0	606
68	669	703	45	32	209	213	1	1	1873
69	268	264	10	12	79	84	0	1	718
70	91	77	3	4	39	37	0	0	251
71	308	278	15	2	78	94	2	0	777
72	143	132	5	7	46	41	0	1	375
73	129	101	2	5	30	34	2	0	303
74	81	131	5	1	26	26	0	0	270
75	196	189	13	6	74	55	1	0	534

TABLE 14 (continued)

PLANT NUMBER	NON CROSSOVERS		CROSSOVERS IN REGION						TOTAL
			1		2		1, 2		
76	85	111	5	4	28	30	0	0	263
78	97	104	4	5	27	31	0	0	268
80	217	241	10	6	59	67	0	0	600
81	97	80	4	2	18	25	0	0	226
82	135	136	2	4	35	53	0	0	365
83	242	248	11	11	99	64	0	0	675
85	145	161	7	10	39	41	1	4	408
87	96	107	5	2	29	34	0	0	273
88	86	77	7	4	21	21	0	0	216
89	54	45	1	2	13	12	0	0	127
90	219	218	8	9	60	60	0	0	574
92	130	123	10	3	44	32	0	0	342
95	37	43	4	1	12	12	0	0	109
96	73	67	3	3	36	25	0	0	207
98	170	153	8	9	46	49	0	0	435
99	235	261	8	14	81	88	0	1	688
Total	20,178		870		5919		39		27,006

TABLE 15

Crossover data, female gametes, family 4003.
(Tiller ears excluded).

PLANT	NON-CROSSOVERS		CROSSOVERS IN REGION						TOTAL
			1		2		1, 2		
2	116	96	8	4	24	41	3	2	294
4	187	161	5	4	41	41	1	1	441
5	337	333	10	12	74	72	4	2	844
7	342	324	17	13	99	96	0	0	891
9	307	296	5	6	100	102	1	0	817
10	210	205	6	5	52	33	1	0	512
11	185	178	1	4	40	61	0	0	469
13	292	312	4	6	65	92	0	0	771
15	161	200	6	2	46	61	0	0	476
16	390	360	6	5	65	83	1	1	911
17	206	155	2	4	36	51	0	0	454
18	254	235	6	7	62	77	0	0	641
19	188	221	5	8	55	53	0	0	530
20	331	330	10	9	84	81	1	1	847
24	216	183	3	2	47	38	0	0	489
27	316	328	11	6	109	107	0	0	877
28	464	471	15	15	105	126	0	0	1196
29	448	470	5	8	100	96	0	0	1127
30	262	247	4	3	61	63	0	0	640
31	273	271	7	9	73	79	0	1	713
32	250	258	8	9	50	52	0	0	627
33	221	218	7	6	48	51	0	0	551
34	362	355	11	17	95	73	2	0	915
40	385	377	10	14	89	86	0	0	961
44	406	386	7	11	90	92	0	0	992
46	354	345	14	14	117	112	0	1	957
47	325	335	14	19	102	94	0	0	889
52	358	344	7	15	92	94	0	0	910
53	179	162	4	7	31	34	0	0	417
54	406	459	8	11	93	82	0	0	1059
55	182	160	8	10	50	61	0	0	471
58	70	79	6	1	12	18	0	0	186
59	419	425	17	15	80	92	0	1	1049
61	410	426	3	5	89	91	0	0	1024
63	338	328	12	16	87	86	0	0	867
65	296	277	4	3	60	61	1	0	702
66	393	391	15	15	89	96	0	0	999
67	212	201	5	5	46	56	0	0	525
68	157	168	8	8	50	53	0	0	444
69	206	198	5	5	58	53	0	0	525
70	307	315	18	15	114	104	1	1	875
71	439	424	12	18	87	91	0	0	1071
72	469	419	13	18	106	92	0	0	1117
73	305	277	16	11	96	99	0	0	804

TABLE 15 (continued)

PLANT	NON-CROSSOVERS		CROSSOVERS IN REGION						TOTAL
			1		2		1, 2		
75	268	277	9	9	121	103	1	1	789
76	456	379	22	17	98	104	0	0	1076
77	218	244	9	4	67	75	0	0	617
80	301	274	10	6	60	68	0	0	719
81	338	364	11	6	95	97	0	0	911
83	262	247	4	0	71	67	0	0	651
85	337	331	13	11	45	81	1	0	819
86	262	279	2	4	50	45	0	0	642
87	325	380	9	7	77	102	0	0	900
88	404	403	8	7	130	136	0	0	1088
89	306	297	9	8	93	88	0	0	801
90	207	206	5	6	61	66	1	0	552
93	119	107	1	0	16	31	0	0	274
95	157	155	4	6	44	42	1	0	409
96	348	360	9	8	80	98	0	0	903
98	278	266	4	9	67	67	0	0	691
99	389	375	11	13	97	95	0	0	980
100	50	52	1	3	14	13	0	0	133
Total	35,658		1033		9109		32		45,832

Ears without separation of Sh sh (crossovers in C-W_x region)

3	157	148	—	—	44	61	—	—	410
12	95	99	—	—	41	39	—	—	274
14	307	304	—	—	118	99	—	—	828
35	97	108	—	—	30	29	—	—	264
41	384	362	—	—	83	110	—	—	939
42	98	89	—	—	26	20	—	—	233
78	74	65	—	—	33	18	—	—	190
Grand total	38,045		——		10,893		——		48,970

TABLE 16

Crossover percentage in male and female gametes. Family 4003.

PLANT NUMBER	*C-Sh* REGION			*Sh-Wx* REGION			*C-Wx* REGION		
	Male gametes	Female gametes	Difference	Male gametes	Female gametes	Difference	Male gametes	Female gametes	Difference
5	2.7±0.9	3.3±0.3	−0.6±0.9	20.5±2.1	18.0±1.0	2.5±2.3	22.2±2.2	19.9±1.0	2.3±2.4
7	4.1±0.5	3.4±0.3	0.7±0.6	20.2±1.1	21.9±0.9	−1.7±1.4	23.6±1.1	25.3±1.0	−1.7±1.5
10	2.6±0.7	2.3±0.4	0.3±0.8	19.3±1.6	16.8±1.2	2.5±2.0	21.2±1.7	18.8±1.3	2.4±2.1
11	4.0±0.5	1.1±0.5	2.9±0.7	18.2±1.2	21.5±1.3	−3.3±1.8	20.5±1.2	22.6±1.3	−2.1±1.8
13	3.1±0.5	1.3±0.4	1.8±0.7	22.5±1.1	20.4±1.0	2.1±1.5	25.6±1.2	21.7±1.0	3.9±1.6
16	3.0±0.5	1.4±0.3	1.6±0.6	25.6±1.2	16.5±0.9	9.1±1.5	28.6±1.2	17.5±1.0	11.1±1.6
18	5.1±0.4	2.0±0.4	3.1±0.6	19.6±1.0	21.7±1.1	−2.1±1.5	24.1±1.0	23.7±1.1	0.4±1.5
19	5.5±0.7	2.5±0.4	3.0±0.8	22.9±1.7	20.4±1.2	2.5±2.1	28.4±1.8	22.8±1.3	5.6±2.2
20	2.1±0.8	2.5±0.3	−0.4±0.9	26.3±1.8	19.7±1.0	6.6±2.1	28.3±1.9	21.7±1.0	6.6±2.1
24	0.4±0.8	1.0±0.5	−0.6±0.9	13.8±1.8	17.4±1.3	−3.6±2.2	14.2±1.9	18.4±1.3	−4.2±2.3
27	2.6±0.7	1.9±0.3	0.7±0.8	21.1±1.7	24.6±0.9	−3.5±1.9	23.8±1.8	26.6±1.0	−2.8±2.1
28	2.7±0.5	2.5±0.3	0.2±0.6	24.4±1.2	19.3±0.8	5.1±1.4	26.7±1.3	21.8±0.8	4.9±1.5
29	1.6±0.6	1.2±0.3	0.4±0.7	20.9±1.4	17.4±0.8	3.5±1.6	21.9±1.5	18.5±0.9	3.4±1.7
30	3.9±0.6	1.1±0.4	2.8±0.7	22.0±1.3	19.4±1.1	2.6±1.7	25.0±1.4	20.5±1.1	4.5±1.8
33	2.1±0.4	2.4±0.4	−0.3±0.6	20.7±0.8	18.0±1.2	2.7±1.4	22.8±0.9	20.3±1.2	2.5±1.5
34	4.2±0.9	3.3±0.3	0.9±0.9	19.9±2.2	18.6±0.9	1.3±2.4	24.1±2.3	21.4±1.0	2.7±2.5
40	4.1±0.8	2.5±0.3	1.6±0.9	15.7±1.9	18.2±0.9	−2.5±2.1	19.8±2.0	20.7±0.9	−0.9±2.2
44	2.1±0.9	1.8±0.3	0.3±0.9	27.5±2.0	18.3±0.9	9.2±2.2	29.5±2.1	20.2±0.9	9.3±2.3
46	5.3±0.5	3.0±0.3	2.3±0.6	19.6±1.1	24.0±0.9	−4.4±1.4	24.6±1.2	26.9±0.9	−2.3±1.5
47	1.9±0.8	3.7±0.3	−1.8±0.9	25.8±2.0	22.0±0.9	3.8±2.2	27.8±2.0	25.8±1.0	2.0±2.2
52	2.5±0.4	2.4±0.3	0.1±0.5	23.4±1.0	20.4±0.9	3.0±1.3	25.4±1.1	22.9±1.0	2.5±1.5
53	2.5±0.7	2.6±0.5	−0.1±0.9	24.8±0.6	15.6±1.4	9.2±1.5	27.3±1.7	18.2±1.4	9.1±2.2
54	2.2±0.5	1.8±0.3	0.4±0.6	22.9±1.1	16.5±0.9	6.4±1.4	25.0±1.2	18.3±0.9	6.7±1.5
55	4.8±0.4	3.8±0.5	1.0±0.7	19.9±1.0	23.6±1.3	−3.7±1.6	24.5±1.0	27.4±1.3	−2.9±1.6

TABLE 16 (continued)

PLANT NUMBER	C-S_h REGION			S_h-W_x REGION			C-W_x REGION		
	Male gametes	Female gametes	Difference	Male gametes	Female gametes	Difference	Male gametes	Female gametes	Difference
58	3.2±0.5	3.8±0.7	−0.6±0.9	21.4±1.2	16.1±2.1	5.3±2.4	24.2±1.3	19.9±2.1	4.3±2.5
61	2.8±0.4	0.8±0.3	2.0±0.5	23.2±1.0	17.6±0.9	5.6±1.3	25.7±1.1	18.4±0.9	7.3±1.4
63	3.9±0.8	3.2±0.3	0.7±0.9	19.7±1.9	20.0±1.0	−0.3±2.1	23.6±1.9	23.2±1.0	0.4±2.1
65	3.4±0.6	1.1±0.4	2.3±0.7	25.5±1.3	17.4±1.1	8.1±1.7	28.9±1.4	18.2±1.1	10.7±1.8
66	4.3±0.5	3.0±0.3	1.3±0.6	16.8±1.2	18.5±0.9	−1.7±1.5	21.1±1.2	21.5±0.9	−0.4±1.5
68	4.2±0.3	3.6±0.5	0.6±0.6	22.6±0.7	23.2±1.3	−0.6±1.5	26.6±0.7	26.8±1.4	−0.2±1.6
69	3.2±0.4	1.9±0.4	1.3±0.6	22.8±1.1	21.1±1.2	1.7±1.6	25.8±1.1	23.0±1.3	2.8±1.7
70	2.8±0.8	4.0±0.3	−1.2±0.9	30.3±1.8	25.1±0.9	5.2±2.0	33.1±1.9	28.7±1.0	4.4±2.1
71	2.7±0.4	2.8±0.3	−0.1±0.5	24.6±1.0	16.6±0.9	8.0±1.3	26.8±1.1	19.4±0.9	7.4±1.4
72	3.5±0.6	2.8±0.3	0.7±0.7	23.5±1.5	17.7±0.8	5.8±1.7	26.4±1.5	20.5±0.9	5.9±1.7
73	3.0±0.7	3.4±0.4	−0.4±0.8	21.8±1.6	24.3±1.0	−2.5±1.9	23.4±1.7	27.6±1.0	−4.2±2.0
75	3.7±0.5	2.5±0.4	1.2±0.7	24.3±1.2	28.6±1.0	−4.3±1.6	27.7±1.3	30.7±1.0	−3.0±1.6
76	3.4±0.7	3.6±0.3	−0.2±0.8	22.1±1.7	18.8±0.9	3.3±1.9	25.5±1.8	22.4±0.9	3.1±2.0
80	2.7±0.5	2.2±0.4	0.5±0.7	21.0±1.2	17.8±1.0	3.2±1.6	23.7±1.2	20.0±1.1	3.7±1.6
81	2.7±0.3	1.9±0.3	0.8±0.4	19.0±1.9	21.1±0.9	−2.1±2.1	21.7±2.0	22.9±1.0	−1.2±2.2
83	3.3±0.5	0.6±0.4	2.7±0.7	24.1±1.1	21.2±1.1	2.9±1.6	27.4±1.1	21.8±1.1	5.6±1.6
85	5.4±0.6	2.9±0.4	2.5±0.7	20.8±1.4	15.5±1.0	5.3±1.7	23.8±1.5	18.3±1.0	5.5±1.8
87	2.6±0.7	1.8±0.3	0.8±0.8	23.1±1.7	19.9±0.9	3.2±1.9	25.6±1.8	21.7±1.0	3.9±2.1
88	5.1±0.8	1.4±0.3	3.7±0.9	19.4±1.9	24.4±0.8	−5.0±2.1	24.5±2.0	25.8±0.9	−1.3±2.2
89	2.4±1.1	2.1±0.4	0.3±1.2	19.7±2.5	22.6±1.0	−2.9±2.7	22.0±2.6	24.7±1.0	−2.7±2.8
90	3.0±0.5	2.2±0.4	0.8±0.7	20.9±1.2	23.2±1.2	−2.3±1.7	23.9±1.2	25.0±1.2	−1.1±1.7
95	4.6±1.1	2.7±0.5	1.9±1.2	22.0±2.7	21.3±1.4	0.7±3.0	26.6±2.8	23.5±1.4	3.1±3.1
96	2.9±0.8	1.9±0.3	1.0±0.9	29.5±2.0	19.7±0.9	9.8±2.2	32.4±2.1	21.6±1.0	10.8±2.3
98	3.9±0.6	1.9±0.4	2.0±0.7	21.8±1.4	19.4±1.1	2.4±1.8	25.7±1.4	21.3±1.1	4.4±1.8
99	3.3±0.5	2.4±0.3	0.9±0.6	24.7±1.1	19.6±0.9	5.1±1.4	27.8±1.1	22.0±0.9	5.8±1.4

TABLE 17

Repeated pollinations from plant 4003-68.

TASSEL	DATE AUGUST	NON-CROSSOVER		CROSSOVERS IN REGION						TOTAL
				1		2		1, 2		
Main	2	140	176	8	8	57	47	0	0	436
	4	135	182	4	7	46	42	0	0	416
	4	151	140	4	2	56	47	0	1	401
	5	182	205	10	4	40	58	0	0	499
	7	297	341	19	10	119	115	0	0	901
	8	42	51	0	2	15	14	0	0	124
First tiller	7	184	194	11	7	51	57	0	0	504
	8	111	101	5	8	27	35	0	0	287
	9	268	253	20	10	81	81	0	0	713
	10	77	93	4	5	30	27	0	0	236
	12	70	76	6	4	20	17	0	0	193
	13	74	82	2	2	14	21	0	1	196
Second tiller	9	96	75	6	4	25	38	0	0	244
	10	83	93	6	3	23	33	1	1	243
	11	138	143	6	7	43	48	1	1	387
	12	82	90	6	8	25	33	0	0	244
	13	68	82	5	3	28	24	0	0	210
	14	128	155	7	6	35	53	0	1	385
Third tiller	13	143	141	9	7	48	34	0	1	383
	14	26	32	3	3	8	12	0	0	84
	16	134	138	10	6	26	48	0	2	364
	17	155	171	12	9	51	41	0	0	439
Fourth tiller	18	106	117	11	6	28	37	1	0	306
	19	35	38	4	1	14	6	0	0	98

Table 18

Repeated pollinations from plant 4013-2.

TASSEL	DATE	NON-CROSSOVER	CROSSOVERS IN REGION 1	CROSSOVERS IN REGION 2	CROSSOVERS IN REGION 1, 2	TOTAL
Main	July 25	23 24	0 2	9 8	0 0	66
	July 27	86 96	5 4	30 34	0 0	255
	July 28	153 153	8 4	51 72	0 1	442
	July 29	49 57	2 2	16 23	1 0	150
	July 30	57 58	3 2	30 44	0 0	194
	July 31	41 35	3 4	18 22	0 0	123
	Aug. 1	34 48	3 4	18 27	0 0	134
First tiller	Aug. 5	76 59	4 3	35 34	0 0	211
Second tiller (transplanted)	Aug. 7	49 50	2 0	22 30	0 1	154
	Aug. 9	34 39	1 1	13 16	1 0	105

Table 19

Repeated pollinations from plant 4017-5.

TASSEL	DATE	NON-CROSSOVER	CROSSOVERS IN REGION 1	CROSSOVERS IN REGION 2	CROSSOVERS IN REGION 1, 2	TOTAL
Main	July 25	130 103	7 6	18 24	0 0	288
	July 27	116 121	9 12	39 39	0 1	337
	July 28	115 106	1 16	36 34	0 0	308
	July 31	99 102	9 3	31 14	0 0	258
	Aug. 2	96 107	2 4	24 15	0 0	248
First tiller	Aug. 3	152 145	12 7	35 38	0 0	389
Second tiller (transplanted)	Aug. 7	90 73	5 2	25 26	1 0	222
	Aug. 10	76 64	0 0	14 19	0 1	174
Third tiller	Aug. 10	77 66	4 4	21 10	0 2	184
	Aug. 12	142 151	9 5	32 27	0 0	366
Fourth tiller	Aug. 14	211 176	6 15	55 41	0 0	504
	Aug. 16	67 56	4 1	20 11	0 0	159

TABLE 20

Repeated pollinations from plant 4017-21.

TASSEL	DATE	NON-CROSSOVER	CROSSOVERS IN REGION			TOTAL
			1	2	1, 2	
Main	July 26	82 86	1 5	20 14	0 0	208
	July 27	127 106	2 5	29 21	0 0	290
	July 29	137 146	7 9	32 44	0 0	375
	July 30	94 102	3 3	26 22	0 0	250
	Aug. 2	27 32	2 1	7 4	0 0	73
First tiller	Aug. 3	83 91	4 3	26 24	0 0	231
	Aug. 7	77 87	4 1	25 25	0 1	220
Second tiller (transplanted)	Aug. 7	54 66	0 1	19 26	0 0	166
	Aug. 9	57 57	3 1	18 17	0 0	153
	Aug. 10	75 74	2 4	16 19	0 0	190
	Aug. 11	106 124	9 3	36 33	0 0	311
Third tiller	Aug. 16	106 150	3 4	31 49	0 1	344

TABLE 21

Crossover data, first tassel and first tiller tassel, family 4003.

PLANT	FIRST TASSEL					FIRST TILLER TASSEL				
	Non-crossover	Crossovers in region			Total	Non-crossover	Crossovers in region			Total
		1	2	1, 2			1	2	1, 2	
13	88 70	2 2	16 18	0 0	196	66 62	3 3	22 28	0 0	184
16	112 111	5 4	40 33	0 0	305	74 105	5 3	35 36	0 0	258
18	181 185	12 12	34 51	1 0	476	128 105	4 10	32 36	0 1	316
23	69 85	6 2	27 22	0 0	211	114 162	7 4	51 41	0 1	380
28	110 130	4 5	51 34	1 0	335	65 78	1 3	21 21	0 0	189
41	90 101	5 3	29 43	0 0	271	74 56	4 3	20 19	0 0	176
46	94 121	12 7	27 29	1 0	291	115 138	6 7	41 24	0 0	331
54	165 187	7 2	66 41	0 0	468	67 63	3 2	15 25	0 0	175
55	114 117	10 9	35 33	0 0	318	95 94	5 7	18 23	0 0	242
58	106 99	2 4	25 35	0 1	272	103 95	3 7	26 27	0 0	261
61	80 103	4 2	29 27	0 0	245	107 102	3 4	31 37	0 1	285
65	48 56	1 2	21 15	0 0	143	108 122	7 6	41 43	0 0	327
66	101 89	4 2	23 18	0 0	237	149 139	10 10	31 30	0 0	369
68	140 176	8 8	57 47	0 0	436	184 194	11 7	51 57	0 0	504
69	66 70	3 4	22 27	0 0	192	88 84	2 4	24 24	0 0	226
73	60 49	1 2	17 17	1 0	147	69 52	1 3	13 17	1 0	156
83	67 61	2 2	26 19	0 0	177	85 86	5 6	40 23	0 0	245
85	67 74	3 3	25 18	0 0	190	78 87	4 7	14 23	1 4	218
99	127 144	6 5	50 50	0 0	382	108 117	2 9	31 38	0 1	306
Total	3913	177	1197	5	5292	3818	191	1129	10	5148

TABLE 22

Crossover data, first ear and first tiller ear, family 4003.

PLANT	FIRST EAR					FIRST TILLER EAR				
	Non-crossovers	Crossovers in region			Total	Non-crossovers	Crossovers in region			Total
		1	2	1, 2			1	2	1, 2	
7	195 173	8 5	55 49	0 0	485	181 159	7 7	56 61	0 0	471
11	185 178	1 4	40 61	0 0	469	163 146	5 6	40 53	0 0	413
13	130 157	1 1	34 38	0 0	361	118 127	3 5	34 29	0 0	316
16	162 159	2 1	36 40	0 0	400	219 204	3 1	53 55	0 0	535
17	206 155	2 4	36 51	0 0	454	157 171	3 6	37 55	0 0	429
19	106 123	4 6	25 24	0 0	288	61 69	0 0	12 16	0 0	158
24	216 183	3 2	47 38	0 0	489	165 139	3 3	58 49	0 0	417
31	172 165	3 7	43 45	0 0	435	132 149	4 3	35 43	0 0	366
33	221 218	7 6	48 51	0 0	551	218 227	1 3	44 56	0 0	549
34	198 179	6 9	58 43	2 0	495	59 73	2 2	27 13	0 0	176
46	191 167	7 5	50 61	0 1	482	175 164	5 6	59 53	0 0	462
52	171 173	3 9	41 45	0 0	442	187 165	0 2	40 57	0 0	451
53	179 162	4 7	31 34	0 0	417	158 140	3 1	39 48	0 0	389
54	229 233	4 6	42 46	0 0	560	196 195	7 5	38 37	0 0	478
61	216 234	2 2	47 43	0 0	544	47 66	0 3	12 17	0 0	145
63	182 187	8 8	54 52	0 0	491	160 142	9 12	52 53	0 0	428
67	146 128	4 3	33 33	0 0	347	175 191	3 4	63 48	0 0	484
68	157 168	8 8	50 53	0 0	444	216 214	13 14	67 62	0 1	587
69	206 198	5 5	58 53	0 0	525	144 165	7 7	41 52	0 0	416
80	122 110	5 3	25 26	0 0	291	54 53	1 2	19 17	0 0	146
85	137 135	6 5	16 30	0 0	329	131 111	3 7	30 31	0 0	313
99	198 185	4 6	43 42	0 0	478	158 161	2 9	49 48	1 0	428
Total	7695	209	1870	3	9777	6504	192	1858	2	8557

EFFECT OF THE WAXY GENE IN MAIZE POLLEN,— A REPLY TO CRITICISMS[1]

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It was independently discovered and coincidentally reported by DEMEREC (1924) and by BRINK and MACGILLIVRAY (1924) that maize pollen grains carrying the waxy gene give a unique reaction with the iodine-potassium-iodide solution commonly employed in detecting starch. LONGLEY (1924) has also reported this phenomenon. In a recent paper, KIESSELBACH and PETERSEN (1925) question the validity of our observations and conclusions.

In our original papers we showed that pollen of flint, dent, pop and sweet varieties of maize contain reserves giving the well known blue reaction characteristic of starch when tested with iodine. On the other hand, the contents of pollen grains produced by homozygous waxy plants fail to give this blue color when similarly treated, but stain a dull yellow passing to reddish under conditions favoring gradual concentration of the test solution. It was found, moreover, that individuals heterozygous for the waxy character produce both types of pollen grains, namely, blue-staining and yellowish-staining, in approximately equal numbers. This significant fact we took to indicate an immediate effect of the segregation of the non-waxy–waxy allelomorphs.

KIESSELBACH and PETERSEN have been unable to demonstrate this difference in the nature of waxy and non-waxy pollen and hold that it does not exist. Their observations and conclusions may be briefly summarized as follows: (1) Normally-developed mature pollen from waxy as well as from non-waxy races of maize gives the starch reaction with iodine. (2) The iodine solution does not serve to differentiate the content of the pollen grains of waxy and non-waxy maize. (3) The walls of the immature pollen of all varieties assume a reddish color with iodine which in older material is obscured by the dark stain of the starch within the grain. (4) Starch deposition in the grains of a single anther is sometimes not completed simultaneously, resulting in a transition stage in which some of the pollen stains reddish and some blue. (5) This latter condition was mistaken by the present investigators as evidence of segregation of the non-waxy–waxy genes.

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It was made clear, we thought, at the time our discovery was reported, that the difference in the characters of the male gametophytes occasioned by the non-waxy-waxy allelomorphs rests upon a distinctive color reaction with iodine, of the two types of pollen *reserves*. It was pointed out that such difficulties in observation as were occasioned by the pollen-grain wall may be readily overcome by resort to the simple expedient of applying enough pressure to the cover-glass of the mount to rupture the grains and force out a portion of their contents. If attention is then focussed upon the granular inclusions of the cytoplasm, a difference in these materials from non-waxy and waxy gametophytes is readily apparent. In the case of pollen from homozygous non-waxy plants numerous small, mostly rod-shaped and ovate, blue-staining starch grains are present; the corresponding bodies from waxy pollen, which are similar in size but somewhat more irregular in shape, fail to give this blue reaction with iodine. If the preparation is allowed to stand unsealed for an hour or so, a little fresh solution being added from time to time, these waxy-type granules assume a reddish color. Plants segregating for waxy produce these two sorts of pollen with equal frequency.

Since this phenomenon was first reported, one of us (BRINK) has confirmed the difference in the nature of the reserve carbohydrates in non-waxy and waxy pollen by separating these substances in relatively pure form and studying their color reactions *in vitro*. Freed from both the spore coat and the cytoplasm in which they are found, it can be demonstrated in an unequivocal manner that the two types of reserves, when stained with iodine, provide a reliable basis for distinguishing non-waxy and waxy pollen. Although they do not stain as readily, these constituents of the pollen can be differentiated quite as clearly as the corresponding endosperm reserves. If small amounts of the granular carbohydrate reserves separated from the pollen of non-waxy and waxy plants are heated to boiling with water and a few drops of iodine added to the suspensions after cooling, the color reactions obtained are practically identical with those given by the corresponding materials from the endosperm. Following RIDGWAY'S (1912) standards, the color of the non-waxy preparation is Helvetia Blue, while that of the waxy preparation is Rood's Violet.

We cannot give credence to the statement by KIESSELBACH and PETERSEN that mature pollen of waxy and non-waxy races alike gives the typical starch reaction with iodine, nor can we let their observation that no difference is shown in the color reaction of the contents of the two sorts of crushed grains pass unchallenged. Of course, one may stain both types of pollen so heavily that they will appear black simply through failure to transmit light. In getting a satisfactory differentiation some discrimination as to strength of solution and time of staining must be exercised, to be sure, but the conditions of a satisfactory test are not difficult of fulfillment.

The argument that the difference which we have taken to indicate the effect of segregation, is a transitory condition, not only fails to account for the 1 : 1 distribution of the two types of pollen, but is not founded on fact. We have samples of pollen, collected at the time of dehiscence from non-waxy and waxy plants, which have been stored in the laboratory over calcium chloride for some eight months, which are as readily distinguished from each other now as when they were fresh.

As proved by the breeding results we have found the iodine reaction at pollinating time a uniformly dependable means of separating homozygous non-waxy from heterozygous plants in progenies comprising these genotypes. There is little ground for questioning the validity of a test which satisfies this criterion.

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THE MULTIPLE STOCK "XPLE" AND ITS USE

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Both in the construction and in the use of a map that shows the relative location of the genes contained in a given chromosome, it is necessary to have a table of corrections which gives for each two loci the amount by which the number of units of map-distance is greater than the percentage number of recombinations that is directly observed in an experiment. A few examples will make this clear. The percentage of recombinations for two linked characters is usually found by a "backcross" experiment, in which a female heterozygous for the two pairs of characters is mated to a male homozygous for the recessives of both pairs. Thus, if the recessive scute (lack of certain bristles from the scutellum) is crossed to the recessive echinus (a larger eye with coarser hairs on it) the F$_1$ female will be wild-type, for each parent contributes the recessive of one pair and the dominant wild-type allelomorph of the other pair $\left(\dfrac{s_c \quad +^{e_c}}{+^{s_c} \quad e_c}\right)$. This F$_1$ female is then mated to a male homozygous for both scute and echinus. The offspring are found to be of four classes. Two, scute and echinus, respectively, represent the original combinations of the four characters dealt with (scute with not-echinus, and not-scute with echinus). Two other classes are scute echinus and wild-type. These represent new combinations of the two pairs of characters that entered the cross (scute with echinus and not-scute with not-echinus). The percentage of recombination is found by dividing the sum of the two recombination classes by the total number in all classes, and multiplying by a hundred. A representative scute echinus backcross experiment (data from table 5) gave: $s_c = 9805$, $e_c = 9575$, $s_c\ e_c = 686$, $+ = 720$. The total recombinations were 1406, which is 6.8 percent of the total of 20,786 flies. With the crossveinless wing-character, scute gave 16.3 percent of recombination. In a given linkage group each two pairs of characters have a characteristic recombination percent.

The recombinations of linked characters is explained by "crossing over" of the chromosomes. In the cells that are about to give rise to eggs, the chromosome carrying scute comes to lie closely side-by-side with the homologous chromosome carrying vermilion. At some point or points

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along the synapsed chromosomes the two chromosomes break across, and then rejoin in such a way that the part of each that lies to the left of the break is united with the part of the other that lies to the right of the break. Such points of crossing over may occur anywhere along the chromosome, and whenever one falls between the loci occupied by scute and echinus it gives rise to crossover chromosomes, one of which carries both mutant genes and the other of which carries neither. The individuals that come from the crossover chromosomes show recombinations of characters. The further apart the loci of two characters are, the more frequently will crossovers fall between them, and the higher will be the percentage of recombinations.

But if the loci of two characters are far enough apart (which is true for scute and crossveinless) two crossovers sometimes fall between them. In such an event, the genes that were together originally remain together in spite of the double crossing over between them. A section of chromosome lying between two genes is replaced by a section from the other homologue. No recombination of the characters is produced, and for each such case the total number of crossovers is increased by two, while the total number of recombinations of the linked characters is not increased at all. Thus the number of recombinations corresponds to the full number of crossovers only in case the two loci considered are so close together that no double crossing over occurs between them. In constructing the maps it is necessary to know the full number of crossovers (doubles counting as two, triples as three crossovers) that occur between the loci that are to be mapped. A unit of map distance corresponds to a section of chromosome of such length that one crossover falls within it for each average hundred gametes. In constructing the map of the first chromosome which is given as the base line in figure 1, scute and echinus were placed 6.8 units apart, which means that in the data on which that map was based (table 5) 6.8 crossovers between scute and echinus occurred for each hundred gametes. None of these were doubles, since scute and echinus are too close together to allow double crossing over between them. Scute and forked are there mapped as 62.0 units apart. Scute and forked are so far apart that not only double, but triple and quadruple crossing over has been found to occur between them. In constructing such maps it is first necessary to determine experimentally the amounts by which the various crossing over values exceed the directly observed recombination percents.

The experimental determination of the amount by which crossing over exceeds recombination is made through use of loci lying between the two loci in question, and which are so close together that no double crossing over occurs between succeeding ones. Thus, the number of double crossovers occurring between scute and crossveinless can be determined through use of the intermediate locus echinus, the scute-echinus interval and the echinus-crossveinless interval being free of double crossing over. An F_1 female from the cross between scute crossveinless and echinus $\left(\frac{s_r \quad +^{'c} \; c_c}{+^{sc} \; e_c \; +^{cv}}\right)$ is backcrossed to a scute echinus crossveinless male. All double crossovers that occur between scute and crossveinless are easily detectable, since they result in $s_c \; e_c \; c_v$ and in wild-type flies, the number of which can be counted. In the data of table 5 there were 0.03 percent such doubles, which gives a difference of 0.06 between crossing over and recombination. This difference is very slight, and it is partly because it is so slight (and partly from direct tests) that it is certain that no doubles occur within either of the two smaller sections. To determine the difference for loci as far apart as scute and forked requires the simultaneous use of several intermediate loci.

The selection of these intermediate loci is a problem of no little difficulty, and it is only recently that mutant loci of suitable characteristics have become available. First, only mutant characters that are separable from the wild-type without error may be used. This requires a character that does not fluctuate greatly and for which the gap between the wild-type and the fluctuant closest to the wild-type is sharp. Second, the mutants must have practically normal viability, fertility and productivity. Some mutants that themselves have normal viability show great mortality if they are combined with certain other mutants. Thus, sable has been found to greatly decrease the viability of multiple stocks. Third, those used together must be sharply separable from one another, and every class possible from combination of the constituent characters must be sharply separable from every other class. Thus, ruby and garnet eye colors cannot be used together, nor singed and forked bristles. From the suitable mutants as small a number should be selected as possible and still have the number of doubles between successive loci either zero, or negligibly small (less than one double per 2000 flies), or small and of an amount known from other experiments.

In 1916 the best selection for the scute to forked interval was echinus, cut, vermilion and garnet. To carry out backcrosses a multiple stock containing these recessives together with scute and forked was necessary. The $s_c \; e_c \; c_t \; v \; g \; f$ multiple recessive was made by BRIDGES and called "Xple" (X-chromosome multiple). The Xple stock was used in the COLUMBIA laboratory in finding the locus of several sex-linked mutants, and was sent to various workers elsewhere, who used it in studying the effect upon crossing over of several environmental and genetic agencies. Thus, PLOUGH (1921) used Xple in experiments that confirmed the finding of BRIDGES (1915) that the age of the mother made little or no difference in the crossing over frequencies for the X chromosome. By use of the same stock PLOUGH showed that heat treatment was likewise without any certain effect. GOWEN and GOWEN (1923) used Xple to show that a third-chromosome recessive gene suppressed all crossing over in the X chromosome. MOHR (1923b) used Xple in testing the length of the deficient section due to notch 8, and in showing that crossing over outside of the deficient section itself is not affected. Mrs. WARD (1923) used Xple to test the effect upon crossing over in the X chromosome of curly, which suppressses nearly all crossing over in the second chromosome. The data indicated that curly influences crossing over in the X to a slight extent. Mohr (1923a) used Xple in finding the locus of a semilethal character chlorotic, whose locus is slightly to the left of scute.

In table 1 is given a summary of the data involving Xple. In table 2 are given the data secured by MOHR in the cross of notch 8 by Xple. The

TABLE 1

Summary of data involving $\frac{s_c\ (1)\ e_c\ (2)\ c\ (3)\ v\ (4)\ g\ (5)\ f}{+\quad +\quad +\quad +\quad +\quad +}$.

SOURCE OF DATA	CROSSOVER REGIONS										
	0	1	2	3	4	5	1,2	1,3	1,4	1,5	2,3
PLOUGH '21	2,216	204	575	521	393	424	17	44	56	60	25
MOHR '23b; ♂♂	434	56	131	135	57	55	..	8	6	4	4
MOHR '23b; ♀♀*	659	73	193	175	105	111	..	5	11	7	5
Total	3,309	333	899	831	555	590	17	57	73	71	34

TABLE 1 (continued)

SOURCE OF DATA	CROSSOVER REGIONS											
	2,4	2,5	3,4	3,5	4,5	1,2,3	1,2,5	1,3,5	1,4,5	2,3,5	2,4,5	3,4,5
PLOUGH '21	63	77	13	39	10	1	1	1	1	1	1	3
MOHR '23b; ♂♂	7	16	1	7	1		.	.	.	..	..	.
MOHR '23b; ♀♀*	8	22	3	9	.	.	.	..	..	..	..	.
Total	78	115	17	55	11	1	1	1	1	1	1	3

* Data unpublished, kindly supplied by MOHR.

TABLE 2

Data from MOHR (1923 a) *involving* $\begin{array}{ccccccccccc} s_c & + & e_c & (2) & c_t & (3) & v & (4) & g & (5) & f \\ + & N_8 & + & & +_t & & + & & + & & + \end{array}$

CROSSOVER REGIONS												
0	2	3	4	5	2,3	2,4	2,5	3,4	3,5	4,5	2,3,4	2,3,5
897	243	251	162	155	4	25	36	11	28	7	1	2

data of table 2 and the other data involving notch 8 show that the crossing-over relations to the right of echinus are probably not affected by the action of notch 8, and accordingly these data can be used in connection with those of table 1. The combined data give the following percentagesof recombination for the successive sections: s_c-$e_c = 7.9$, e_c-$c_t = 16.4$, c_t-$v = 14.6$, v-$g = 10.6$, g-$f = 12.0$. The map based on these data would be about 62.0 units long from scute to forked, which is slightly longer than the old standard map of 56.5 (BRIDGES 1921). The greatest difference is in section 1 (7.9 instead of 5.5) and in section 2 (16.4 instead of 14.5).

On the basis of the data of table 1 alone the scute-forked map distance is 60.9, while the scute-forked recombination percentage is 45.7. That is, each hundred flies involved 15.2 cases of crossing over that did not give rise to recombinations for scute and forked. Discussion of the other points brought out by these data will be deferred until further data have been presented.

In 1919 the excellent sex-linked recessive mutant "crossveinless" was discovered (BRIDGES 1920), and its locus was found to be about half way between those for echinus and cut. By use of crossveinless the distance of about 15 units between echinus and cut could be broken into two sections of about 8 and 7 units. Each of these sections would be so short that no double crossing over would occur within it, and the number of the doubles occurring between echinus and cut could be accurately determined by direct experiment. An X-multiple that includes crossveinless in addition to the others already in use would be much more effective. Also, two other mutants had been discovered by BRIDGES that offered improvements over those in the old Xple. The mutant cut in the old Xple was the original cut found by BRIDGES in 1915. An allelomorph of cut, namely cut^6, was found that was more viable than the original cut and that did not affect the eyes, antennae, and abdomen to such an extent as the other allelomorphs. Classification was perfect on the basis of the wing character, the wing being "cut" to a sharp point. Likewise a new allelomorph of garnet, namely, garnet^2, was found, that had a lighter eye

TABLE 3

Backcross data $\frac{s_c\ (1)\ e_c\ (2)\ c_v\ (3)\ c^6\ (4)\ v\ (5)\ g^2\ (6)\ f}{+\quad +\quad +\quad +\quad +\quad +\quad +}$.

CULTURE NUMBER	CROSSOVERS IN REGION																									
	0		1		2		3		4		5		6		1,2		1,3		1,4		1,5		1,6		2,3	
	s_c e_c c_v c_t v g f	+	s_c	e_c c_v c_t v g f	s_c e_c	c_v c_t v g f	s_c e_c c_v	c_t v g f	s_c e_c c_v c_t	v g f	s_c e_c c_v c_t v	g f	s_c e_c c_v c_t v g	f	s_c c_v c_t v g f	e_c	s_c c_t v g f	e_c c_v	s_c v g f	e_c c_v c_t	s_c g f	e_c c_v c_t v	s_c f	e_c c_v c_t v g	s_c e_c v g f	c_v c_t
2	46	55	6	6	7	7	9	15	16	19	12	15	5	10	..	1	..	..	1	1	.	2	1	1	..	..
4	65	58	16	3	11	9	9	9	16	13	12	6	11	9	..	..			..	2	2	..	.	.	..	.
6	42	54	16	7	10	9	12	6	17	14	13	13	9	8	..	..	1	1	2	3	..	5	1	1	..	..
7	73	87	11	7	24	12	15	13	21	18	12	10	9	13	.	..	..	..	2	..	..	..	.	1	1	..
9	71	109	21	13	23	18	20	26	26	34	19	21	9	13				..	1	.	4	..	4	5	..	..
11	28	92	17	6	15	14	13	13	17	28	19	19	4	16			.	.	.	2	.	4	1	1	.	.
12	86	133	23	13	20	22	19	18	37	25	14	17	13	17		.	1	.	..	2	1	1	.	2	.	..
13	61	78	9	9	15	4	16	7	24	23	11	19	15	15		..	.	.	2	4	3	..	.	1	.	..
14	19	29	7	4	5	7	6	3	2	10	7	12	5	8	.	..	.	.	..	..	1	..	1	1	..	..
16	70	104	11	14	10	11	11	16	23	25	12	14	14	17	1	.	..	.	1	2	2	3	1	2	..	..
Totals	561	799	127	82	140	113	130	126	199	209	121	146	94	126	1	1	2	1	9	16	13	15	9	15	1	0
	1360		209		253		256		408		267		220		2		3		25		28		24		1	

TABLE 3 (continued)

CULTURE NUMBER	CROSSOVERS IN REGION																									
	2,4		2,5		2,6		3,4		3,5		3,6		4,5		4,6		5,6		1,4,5		1,4,6		2,4,6		3,5,6	
	s_c e_c v g f	c_v c_t	s_c e_c g f	c_v c_t v	s_c e_c f	c_v c_t v g	s_c e_c c_v v g f	c_t	s_c e_c c_v g f	c_t v	s_c e_c c_v f	c_t v g	s_c e_c c_v c_t g f	v	s_c e_c c_v c_t f	v g	s_c c_c c_v c_t v f	g	s_c v	e_c c_v c_t g f	s_c v g	e_c c_v c_t f	s_c e_c v g	c_v c_t f	s_c e_c c_v g	c_t v f
2	2	..	2	1	2	1	1	1	..	.	1	..	2	2	1	2	..	..	..	..	..	..	..	..	..	..
4	..	1	1	1	4	1	.	..	.	..	..	1	3	..	2	1	.	..	.	..	..	..	..	.	.	..
6	2	..	2	1	1	3	1	.	..	1		.	1	.		2	1	..	..	..	..	..	..	..	1	.
7	1	1	3	1	5	1	..	..	..	..	.	1	1	1	5	1	..	1	..	..	..	..	.	..	..	..
9	..	2	2	2	3	1	1	..	..	1	.	..	..	..	1	3	1	..	1	..	2	..	..	..	..	..
11	1	..	1	2	..	2	..	1	1	1	2	..	1	..	2	2	..	.	.	..	..	1	.	..	..	..
12	..	3	2	4	4	1	..	1	3	2	3	1	..	2	4	5		..	..	..	..	.	.	.	..	..
13	2	2	2	..	3	..	..	1	2	1	5	1	..	..	3	..	1	..	..	..	..	..	.	..	..	..
14	..	..	..	..	2	..	..	.	..	..	1	2	..	1	1	3	..	1	..	..	..	..	.	..	..	..
16	1	..	1	1	1	3	.	..	..	..	..	2	..	2	..	1	1	1	.	..	..	..	1	..	..	..
Totals	9	9	16	13	25	13	3	4	6	6	12	8	8	8	19	20	4	3	1	0	2	1	1	0	1	0
	18		29		38		7		12		20		16		39		7		1		3		1		1	

color than the original garnet and that could be separated from the wild-type with greater speed and sureness. A second advantage of garnet^2 over garnet is that the vermilion garnet^2 double recessive is a light yellow-orange color easily separated from garnet^2, while the vermilion garnet double recessive is a darker red-orange that is not strikingly different, especially in older flies, from garnet itself. Accordingly, a new Xple was constructed in 1920 with the formula $s_c\ e_c\ c_v\ c_t{}^6\ v\ g^2f$. This Xple has replaced the old both at the COLUMBIA laboratory and at the other laboratories where the most active work is being carried on.

The new Xple had come from various sources, and included only parts of the old Xple. There had been opportunity for a new set of modifiers of the amount of crossing over to be brought together. It was not anticipated that any of these modifiers would be of striking effect, for the stocks used were known to give at least approximately standard values. To test the condition of the new Xple a backcross was made by OLBRYCHT, whose results are given in table 3.

According to the data of table 3 the recombination percentages for the various sections of the Xple were: s_c-$e_c=9.0$; e_c-$c_v=10.5$; c_v-$c_t=9.2$; c_t-$v=15.9$; v-$g=11.2$; g-$f=10.9$. The length of the map from scute to forked is 66.7 units, which is 10.2 units longer than the standard map. Again the increase was largest at the extreme left end and gradually decreased toward the right until the g-f value was slightly below the old standard. The s_c-f recombination percent was 49.8, which is 16.9 lower than the crossover value.

An examination of the details of table 3 shows a striking characteristic of the pairs of complementary classes that constitute the crossover classes. In a given crossover class the two complementary classes are expected to be equal, since the two classes of gametes from which they come are equal. But if the zygotes of one class have a heavier mortality than the zygotes of the complementary class their inequality appears in the ratio of zygotes. In crossover classes 3 and 4 the complementary classes are very nearly equal (130 : 126 and 199 : 209), but in crossover classes 1 and 6 they are quite unequal (127 : 82 and 94 : 126). In crossover class 1 the heavy mortality of the $e_c\ c_v\ c_t{}^6\ v\ g^2f$ class is due to the summation and interaction of six mutant characters, while the relatively slight mortality of the complementary class is due to the single mutant s_c. In crossover class 4 the mortality of the combination of four mutants $s_c\ e_c\ c_v\ c_t{}^6$ is only slightly greater than that of three mutants $v\ g^2f$; and the same is true in class 3 for $c_t{}^6\ v\ g^2f$ and $s_c\ e_c\ c_v$. In crossover classes 2 and 5 the complementary classes have 2 and 5 mutant characters, and the inequality is intermediate between that due to a 3-4 division and to a 1-6 division. It is to be expected that the 7-0 division, namely, Xple and +, as it exists in the non-crossover class, would give the greatest departure from equality of complementary classes. This is undoubtedly the case, but the above data have been selected in such a way as to exclude cultures in which the inequality of Xple and wild-type was strikingly great. The selection was made in the following manner: About 15 cultures were started originally and after they had been hatching a few days the five cultures in which the inequality between Xple and wild-type was greatest were discarded and the counts were completed only for the 10 cultures in which the classes seemed fairly equal. The justification for this selection lies in the fact that those cultures in which the greatest inequalities exist are those in which the culture conditions are most adverse to normal viability, and in which the disturbing effect of differential viability upon the various crossover classes is greatest. The best single index of the normality of viability conditions is the Xple to wild-type ratio, because the greatest effect is expected there, and because in the above experiment those classes were the non-crossover classes and hence the largest classes present. The correlations between the mortality disturbances in the various classes is large, but not so large that a selection based primarily on the Xple and wild-type classes would give equally normal ratios in the other classes. Hence, in the above data the ratio in the 7-0 division (41.2 : 58.8) departs from equality more than one of the 6-1 divisions ($s_c\ e_c\ c_t^6\ v\ g^2 : f = 42.7 : 57.3$), but not more than the other ($e_c\ c_t^6\ v\ g^2 f : s_c = 39.2 : 60.8$). The difference is slight, and for the primary purpose of the experiment, the determination of recombination percentages and crossover values, the data are more valid than they would be if no selection had been made.

TABLE 4

Ratios of complementary classes from table 3.

DIVISIONS	COMPLEMENTARY CLASSES	RATIO
7–0	$s_c\ e_c\ c_v\ c_t\ v\ g\ f$: wild-type	41.2 : 58.8
6–1	$s_c\ e_c\ c_v\ c_t\ v\ g : f$	42.7 : 57.3
5–2	$s_c\ e_c\ c_v\ c_t\ v : g\ f$	45.3 : 54.7
4–3	$s_c\ e_c\ c_v\ c_t : v\ g\ f$	48.8 : 51.2
3–4	$s_c\ e_c\ c_v : c_t\ v\ g\ f$	50.8 : 49.2
2–5	$s_c\ c_c : c_v\ c_t\ v\ g\ f$	55.3 : 44.7
1–6	$s_c : e_c\ c_v\ c_t\ v\ g\ f$	60.8 : 39.2
0–7	Wild-type: $s_c\ e_c\ c_v\ c_t\ v\ g\ f$	58.8 : 41.2

TABLE 5

Alternated backcross of Xple $\frac{s_c \qquad c_v \qquad v \qquad f}{(1)\ e_c\ (2)\quad (3)\ c^6\ (4)\quad (5)\ g^2\ (6)}$.

CULTURE NUMBERS	0		1		2		3		4		5		6		1,2		1,3		1,4	
	s_c c_v v f	e_c c_t g	s_c e_c c_t g	c_v v f	s_c c_t g	e_c c_v v f	s_c c_v c_t g	e_c v f	s_c c_v g	e_c c_t v f	s_c c_v v g	e_c c_t f	s_c c_v v	e_c c_t g f	s_c e_c c_v v f	c_t g	s_c e_c v f	c_v c_t g	s_c e_c c_t v f	c_v g
1	61	57	6	5	6	11	5	9	10	13	14	9	11	13	..	.	1	..	1	.
2	81	77	3	2	16	13	14	11	26	22	16	16	7	11	.	.	.		..	
3	128	101	13	15	16	15	22	23	27	31	32	23	16	29	..		..	..	..	1
4	110	106	4	8	11	14	10	9	25	15	18	16	21	8	.	.	..	..	..	.
5	122	127	12	12	24	12	28	15	32	25	29	21	24	19			..	..	1	2
6	69	103	8	9	12	24	7	16	19	19	29	12	19	17	.	.	.	.	..	.
7	115	107	13	15	16	19	12	19	31	24	9	16	16	6	..	..	..		2	.
8	58	56	4	6	7	9	15	6	12	22	12	18	10	8	..	..	..	..	.	..
9	78	77	8	12	12	12	20	13	20	22	26	24	28	16	.	..	..	..	.	1
10	77	69	4	9	11	8	10	3	22	20	17	12	11	13	..	..	.	2	1	2
11	75	61	8	3	11	12	9	10	24	17	9	14	10	9	..	.	..	..	1	1
13	85	90	11	14	18	21	8	15	29	20	16	16	17	10	..	..	.		1	.
15	86	76	9	6	18	10	16	10	21	22	21	11	14	9	..	..	.		1	.
16	52	80	16	14	7	8	4	8	19	15	12	13	13	21	.	..	..	.	1	.
Totals	1197	1187	119	130	185	188	179	167	317	287	251	221	217	189	..	..	1	2	9	7
(A) xx	980	922	97	94	144	134	112	160	239	237	140	175	169	158	1	..	1	.	6	9
(B) xxy	767	748	61	66	117	114	101	107	177	205	135	130	114	119	1	..	..	3	5	5
(C) xx	421	443	49	65	73	69	69	60	129	119	82	88	92	70	.	.	..	..	2	5
(D) xxy	888	808	74	105	108	152	139	149	240	227	142	135	102	120	1	..	1	1	9	9
(E) xxy	635	558	72	62	96	85	85	97	157	178	113	105	101	74	..	..	1	..	15	6
Grand total	4888	4666	472	522	723	742	685	740	1259	1253	863	854	795	730	3	0	4	6	46	41
	9554		994		1465		1425		2512		1717		1525		3		10		87	

Table 5 (continued)

CULTURE NUMBERS	1,5		1,6		2,3		2,4		2,5		2,6		3,4		3,5		3,6		4,5		4,6	
	sc ec ct f	*cv v g*	*sc ec ct g f*	*cv v*	*sc v f*	*ec cv ct g*	*sc ct v f*	*ec cv g*	*sc ct f*	*ec cv v g*	*sc ct g f*	*ec cv v*	*sc cv ct f*	*ec v g*	*sc cv ct v f*	*ec g*	*sc cv ct g f*	*ec v*	*sc cv f*	*ec ct v g*	*sc cv g f*	*ec ct v*
1	1	1	2	.			.	1		1	1	1	..	.	1	2	2	1	..	..	2	..
2	1		.	1	1			.	1	1	2	3	.	.	2	..	2	1	4	..	3	5
3	..	1	1	1	1		.	2	2	4	2	2			1	3	3	3	1	..	7	3
4	2	..	3	2	.		2	2	3	5	1	5	.		1	.	1	1	1	1	3	4
5	3	4	1	.	1	.	3	..	3	4	4	4	.	1	2	2	3	6	2	2	2	3
6	.	1	3	.	..	.	2	3	3	3	1		..	.	1	..	..	6	4	1	3	4
7	1	1	4	.	.		5	2	6	1	6	3	.	1	.	3	3	5	.	1	2	2
8	1	1	1	1	.	.		1	1	.	2			..	..	..	.		1	..	1	2
9	1	4	1	1	.	..	1		1	1	4	2	.		1	2	1	3	.	4	5	5
10	1	.	1	.	1		..	2	1	5	2	2		.	2	..	2	2	..	..	2	4
11	1		2	3	..				5	.	3	3		.	2	..	1		..	1	2	4
13	3	.	2		.	.		.	1	2	1	5	1	.	2	2	3	1	..	1	2	4
15	..	1	3	3	.		1		3	3	..	5	..	..	2	1	.	.	2	.	1	..
16	..	1	1	1	.		1	1	1	1	5	1	.	..	..	.	1	1	..	1	2	2
Totals	15	15	25	13	4	.	15	14	31	31	34	36	2	2	17	15	22	30	15	13	37	42
(A)xx	12	12	13	18	1	..	4	5	15	19	24	18	3	2	7	10	15	12	5	8	19	16
(B)xxy	13	8	11	6	1	.	11	6	11	10	14	17	1	2	12	10	13	10	4	5	20	12
(C)xx	4	9	7	8	.		7	4	8	7	15	8	1	2	6	3	5	10	6	2	14	10
(D)xxy	13	18	12	10	.	2	5	8	15	21	14	21	4	4	12	10	11	14	6	8	15	15
(E)xxy	12	9	10	14	.	..	10	6	15	8	17	12	1	2	5	3	4	15	1	3	12	13
Grand total	69	71	78	69	6	2	52	43	95	96	118	112	12	14	59	51	70	91	37	39	117	108
	140		147		8		95		191		230		26		110		161		76		225	

TABLE 5 (continued)

	5,6		1,2,6	1,3,5	1,3,6	1,4,5		1,4,6		1,5,6	2,3,4	2,4,5	2,4,6		2,5,6	3,4,6		3,5,6		4,5,6	1,2,3,4	1,2,3,6
CULTURE NUMBERS	*sc* *cv* *v* *g* *f*	*ec* *ct*	*sc* *ec* *cv* *v*	*sc* *ec* *v* *g*	*ec* *cv* *ct* *g* *f*	*sc* *ct* *v* *g*	*cv* *f*	*sc* *ec* *ct* *v*	*cv* *g* *f*	*cv* *v* *g* *f*	*sc* *g*	*ec* *cv* *f*	*sc* *ct* *v*	*ec* *cv* *g* *f*	*ec* *cv* *v* *g* *f*	*sc* *cv* *ct* *v*	*ec* *g* *f*	*sc* *cv* *ct*	*ec* *v* *g* *f*	*sc* *cv*	*g*	*sc* *ec* *cv* *ct* *g* *f*
0																						
2																		1				
3	2																					
4	1	3	1			1																
5		3				1								1							1	1
6		1																				
7		1																		1		
8		1										1		1								
9																						
10	1																					
11	1	1		1		1																
13		1			1	1		1														
15	2	1			1																	
16								1									1					
Totals	7	12	1	1	2	4		2				1		2			1	1		1	1	1
(A)xx	3	5						1	1								1					
(B)xxy	1	2							1		1											
(C)xx	3	2								1					1		1					
(D)xxy	4	4		1	1	1	2	1	2				2						1			
(E)xxy	2	1						1					1			1						
Grand total	20	26	1	2	3	5	2	5	4	1	1	1	2	2	1	1	3	1	1	1	1	
	46		1	2	3	7		9		1	1	1	4		1	4		2		1	1	1

With the exception just noted, there is a regular seriation in the ratios of complementary classes in the above data (see table 4). It is further to be noted that the number of characters has far greater weight than the particular kind. This is due to the fact that in making Xple only such characters were included as were known to have excellent viability.

The inviability effects will be equalized and neutralized to the greatest extent if the number of mutants in complementary classes is equalized as much as possible. This is accomplished by the "alternated backcross" (BRIDGES 1919, 1921), in which half the characters enter from one parent and half from the other, and in which characters consecutive in the map alternate in the homologous chromosomes of the heterozygous female. Thus, the seven characters of the Xple would be divided into 4 and 3 and the parental combinations would be $s_c\ c_v\ v\ f$ and $e_c\ c_t^6\ g^2$. The heterozygous female would be $\frac{s_c \quad +^{sc} \quad c_v \quad +^{c} \quad v \quad +^{g2} \quad f}{+^{sc}\ e_c \quad +^{cv} \quad c_t^6 \quad +^{v} \quad g^2 \quad +^{f}}$, or more simply formulated, $\frac{s_c \qquad c_v \qquad v \qquad f}{(1)\ e_c\ (2)\ (3)\ c_t^6\ (4)\ (5)\ g^2\ (6)}$. The non-crossover complementary classes contain 4 and 3 mutants, and so also do each of the pairs of complementary classes due to crossing over in the six crossover sections. Also, a majority of the pairs of complementary classes due to multiple crossing over are divisions into 4 and 3. (Thus, 1, 3 double crossing over gives the two classes $s_c\ e_c\ v\ f$ and $c_c\ c_t^6\ g^2$). Only a small proportion of the flies would be in 2-5 divisions, while almost no flies would be in 1-6 or 0-7 divisions.

The stocks of $s_c\ c_v\ v\ f$ and $e_c\ c_t^6\ g^2$ were prepared and crossed by BRIDGES and the F_1 wild-type females were backcrossed to Xple males. About 5000 flies of the alternated backcross were raised by OLBRYCHT (table 5, upper section) for direct comparison with the non-alternated results of table 3, and BRIDGES raised some 16,000 others in the course of studying the effect of the Y chromosome on crossing over in the XXY female (table 5, A, B, C, D and E).

In the total of 20,786 flies none was in the 7-0 division, and only 2, or 0.01 percent, were in 6-1 divisions. In the classes in which the division was 5-2, the total flies were 480, or only 2.2 percent. The ratio was 235 : 245, which is practically equality. All of the remaining flies, 20,304 or 97.8 percent, were in 4-3 divisions, and the ratio in the totals was 10,205 : 10,099, which is very close to equality.

In the non-crossover class and each of the single-crossover classes the number of flies was large, and in each case the complementary classes were practically equal. It is thus seen that the alternated-backcross method was successful in distributing the mortality uniformly throughout the population.

The recombination percents for the various sections are s_c-e_c = 6.8, e_c-c_v = 9.6, c_v-c_t = 8.4, c_t-v = 14.7, v-g = 11.0, g-f = 11.4. Only one of these sections, namely, c_t-v, is long enough for double crossing over to occur within it. Experiments in which tan or lozenge was present between cut and vermilion have shown that there is roughly one double crossover between cut and vermilion for each 2000 flies. The map value for c_t-v is therefore 0.1 larger than the observed recombinations percent (14.7 + 0.1 = 14.8). The maps of the X chromosome corresponding to the alternated Xple is given as the base line of figure 1. In comparison with the standard map (BRIDGES 1921) the alternated-Xple map is longer in the sections to the left of vermilion and shorter to the right of garnet. It is important to have a map based solely on alternated-Xple data, since the probability is that for several years the use of alternated Xple will be the standard method for testing crossing over in the X chromosome.

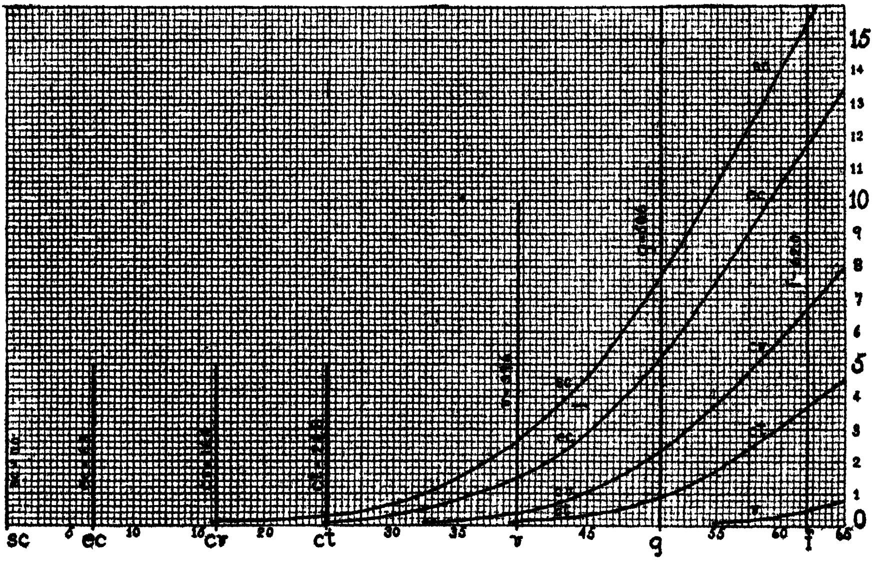

FIGURE 1.—Curves showing difference between map-distance and percentage of recombination for the characters of the alternated-Xple backcross.

The crossover value for scute and forked on the basis of the data of table 5, is 62.0 units. With respect to scute and forked each average hundred gametes was found to consist of 45.91 non-crossovers, 46.37 single crossovers, 7.53 doubles, 0.18 triples and 0.01 quadruples. The total number of s_c-f crossovers is thus $46.37+2\times7.53+3\times0.18+4\times0.01$, which totals 62.01. The percentage of recombinations for scute and forked was 46.55 (the sum of the singles and triples). The number of crossovers per hundred gametes thus exceeded the number of recombinations by 15.46. This difference is also directly obtained as $2\times7.53+2\times0.18+4\times.01=15.46$.

In figure 1 this difference of 15.5 is represented by the height of an ordinate at f. Likewise the s_c-g map distance has been calculated and found to be 50.6, and the corresponding recombination percent is 42.9, the difference of 7.7 being represented by the height of an ordinate at g. For s_c-v the difference is 2.6; for s_c-c_t it is 0.3; for s_c c_v it is 0.1. The smooth curve drawn through these experimentally determined points gives a means of predicting the difference between crossing over and recombinations for scute and any other locus. Thus, to find the expectation for the difference for scute and miniature, the locus of miniature is intercalated in the alternated-Xple map between vermilion and garnet with the same ratio between the *v*-*m* and *m*-*g* intervals as exists in the standard map, (3.1 : 8.3 : : 3.0 : 7.0). The proportionate locus of miniature is thus 3.0 units to the right of vermilion, or at 42.6, and the ordinate at 42.6 cuts the curve from scute at a height of 3.3 units. Similar smooth curves can be drawn through the other points determined by the above experiment, and predictions can thus be made for echinus and any other locus, for crossveinless and any other locus and for cut, vermilion and garnet and other loci.

Curves originating at other points can be intercalated between the curves originating at the points of Xple. Thus, a curve originating at ruby can be intercalated between those originating at echinus and at crossveinless, with the distance from the intercalated curve to the other two curves proportional to the e_c-r_b and the r_b-c_v map distances (2.0 : 6.2).

By extrapolation the curves can be continued beyond the locus of forked until they intersect the ordinates from fused and bobbed. There is also some direct evidence at hand as to the course of the curves beyond forked, so that the extensions need not be pure extrapolation.

The knowledge of the amount of corrections, gained principally from the above data, has been applied in the construction of a standard map with correction curves. This map covers the region to the right of forked, and has intercalated curves originating from the other principal loci besides those included in Xple. It has been published as part of the summary and review of the Drosophila work that appeared in Vol. II of Bibliographia Genetica under the editorship of LOTSY. A map with curves similar to that of figure 1, but based on smaller numbers in the alternated backcross, has appeared in the CARNEGIE INSTITUTION OF WASHINGTON Year Book for 1924.

At present, experiments are under way to extend the information on the course of the curves in the region to the right of forked. These experiments make use of the dominant mutation minute-n, situated at about six units to the right of forked, and the recessive bobbed, situated about 13 units to the right of forked.

In constructing the old standard map (MORGAN and BRIDGES 1916) use was made of experiments that covered the length of the X chromosome with only a few intermediate loci, or that covered particular parts fairly closely. MULLER (1916) devised a method for covering the length of the X chromosome satisfactorily even with mutants that were poorly viable and hard to classify; but the method was so laborious, involving a genetic test of each individual recorded, that he secured only 712 individuals. For some unknown reason the amount of crossing over was abnormally low in MULLER'S data. Most of the later work suggests that a new standard map should be made, and that it would correspond rather closely to the alternated-Xple map presented here.

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THE SEGREGATION OF *OENOTHERA NANELLA-BREVISTYLIS* FROM CROSSES WITH *NANELLA* AND WITH *LAMARCKIANA*[1]

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INTRODUCTION

In earlier papers (DAVIS 1918, 1921) I have given a large body of data on the segregation of *Oenothera brevistylis* from crosses with *Oenothera Lamarckiana*. The results showed that the characters of *brevistylis* are associated and recessive to those of *Lamarckiana* and that the inheritance of *brevistylis* characters from the *Lamarckiana*-like F_1 reciprocal crosses, when selfed, presents in the F_2 a segregation of *brevistylis* and *Lamarckiana* plants in proportions close to the monohybrid ratio of 1 : 3. As would be expected, the same ratio was found in progeny from double reciprocals; that is, from crosses between the two F_1 generations. Finally, backcrosses of the F_1 plants to the recessive *brevistylis* gave segregation of *brevistylis* and *Lamarckiana* approximating the ratio 1 : 1. The results then, in support of earlier conclusions of DE VRIES, consistently showed that the characters of *brevistylis* are inherited as a group according to the simple Mendelian system for a monohybrid.

There were, however, rather constant variations from the expected ratios and always to the disadvantage of *brevistylis* plants, which appeared in numbers not so large as expectations called for, and these smaller proportions seem correlated with a lower seed viability of *brevistylis.* These facts suggested that conditions which reduce the percentage of viable seeds would lower the proportions of *brevistylis* plants. The second paper (DAVIS 1921) gave the results of experiments which proved that malnutrition of developing seeds will kill a much larger proportion of *brevistylis* zygotes or embryos than those of *Lamarckiana*, and malnutrition may therefore be considered as at least one factor affecting the smooth expression of a simple Mendelian inheritance of the group of characters which distinguishes *brevistylis* from *Lamarckiana.*

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[1] Genetical studies on Oenothera XIII. Papers from the Department of Botany, UNIVERSITY OF MICHIGAN, No. 225.

In the cultures, which totalled about 5000 plants, the following well known "mutants" of DE VRIES were identified: 10 *albida*, 2 *elliptica*, 3 *laevifolia*, 3 *lata*, 12 *nanella*, 29 *oblonga*, 1 *rubrinervis*, and 16 *scintillans.* Of these, all but the *albida* and *elliptica* came to flower, and with one exception none of them presented the characters of *brevistylis.* The exception was a plant with the habit and stature of *nanella* (culture 20.76, DAVIS 1921, p. 580, table 3) in an F_2 from the cross *brevistylis* × *Lamarckiana.* The plant had the characters of *nanella* combined with all of the characters of the *brevistylis* complex, namely, short style with deformed or rudimentary stigma lobes, bud cones more stout and with short sepal tips, broader less-pointed bracts, and ovary partly superior and ripening no seeds. This plant, 20.76-34, was named *nanella-brevistylis.*

Nanella-brevistylis is a plant resulting from the association of *brevistylis* characters, which we shall term the short-style complex, with the dwarf stature and habit peculiar to *nanella.* It became then a matter of interest to determine what might happen when *nanella-brevistylis* was crossed to *nanella* and to *Lamarckiana.* From the cross with *Lamarckiana* information might come which would help to determine whether genes for stature and for style length were linked in the same chromosome or whether they lay in different chromosomes as suggested by SHULL (1921). Because the plant of *nanella-brevistylis* produced no seed when pollinated, the cross could be made only with it as the pollen parent.

All of the results reported in this paper are from cultures in which the seeds were forced to complete germination by the following method: After soaking for 24 hours the seeds were submitted, while still in water, to exhaust alternating with pressure of about 30 pounds, 6 to 8 times in a period of from 10 to 24 hours. The exhaust and pressure was effected in an iron case with suitable attachments to a pump operated by an electric motor. By this operation air was drawn out of the pores in the seed coats and water was forced into the interior of the seeds. Following this treatment the seeds were placed on pads of wet filter-paper in Petri dishes and kept shaded at hothouse temperature. Most of the viable seeds sprouted within 4 to 6 days and germination was usually complete after two weeks. The germinating seeds were removed to other Petri dishes and a few days later their seedlings were set in pans. The residue of sterile seeds, after germination was believed to be complete, was tested by pinching each seed with a strong forceps to make sure that none contained embryos. After some experience it is not difficult to judge when germination in a Petri dish is finished; in the cultures here described no ungerminated seeds were found in the residue of sterile structures. The number of seedlings divided by the sum of sterile and fertile seeds gives the percentage of germination.

THE F_1 GENERATION OF *nanella*×*nanella-brevistylis*

The F_1 generation of *nanella* × *nanella-brevistylis* (culture 21.29) was through a plant of typical *nanella* (20.66-34) out of the F_2 of one of my crosses of *brevistylis*×*Lamarckiana*. This plant was pollinated from the original plant of *nanella-brevistylis* (20.76-34). The contents of one large capsule gave 144 seedlings of which 11 were stumpy,—that is, the seedling failed to develop the root from the end of the hypocotyl and soon died. There was a residue of 152 sterile seeds and, therefore, a germination of 48.6 percent. The seed fertility of my cultures of *nanella*, as tested in 1921, 1923 and 1924, was respectively 38.7, 44.3 and 39.6 percent. High mortality in the culture reduced its numbers to 98 plants which came to maturity. All of these were of the long-styled *nanella* type. Thus, the short-style complex of *nanella-brevistylis* is recessive to the long-style complex of *nanella*, as are the characters of *brevistylis* recessive to *Lamarckiana*. Two plants of this F_1 generation (21.29-1 and 21.29-2) were selfed in preparation for the F_2 generations.

THE F_2 GENERATIONS FROM *nanella*×*nanella-brevistylis*

Culture 23.49 was grown from the contents of 5 medium-sized capsules of the F_1 plant 21.29-1. Seedlings numbered 240, of which 49 were stumpy and of course died; 323 sterile seeds remained and the germination was 42.6 percent, which was close to the germination percentage of *nanella*. Mortality among the young plants reduced the culture to 141 rosettes when set in the field, where 7 plants died. Thus, the culture matured 134 plants, of which 20 were *nanella-brevistylis* and 114 *nanella*, a ratio of 1 : 5.7.

Culture 23.50 from the contents of 6 medium-sized capsules of the F_1 plant 21.29-2 produced 94 seedlings, of which 28 were stumpy; there were 377 sterile seeds, a poor germination of 20 percent. The plants set out numbered 55 and all of these matured, giving 10 plants of *nanella-brevistylis* and 45 plants of *nanella*, a ratio of 1 : 4.5.

The segregation of *nanella-brevistylis* from *nanella* was perfectly clear in both cultures, but the ratios of 1 : 5.7 and 1 : 4.5 showed the same departures from the expected ratio of 1 : 3 as appeared in my work with *brevistylis* and *Lamarckiana*. There was deficiency of *nanella-brevistylis*, and it seems probable that some factors work against the full expression of this segregate.

THE F_1 GENERATION OF *Lamarckiana*×*nanella-brevistylis*

The F_1 generation of *Lamarckiana*×*nanella-brevistylis* (culture 21.30) was through a plant (20.10-9) of my line of *Lamarckiana* from DE VRIES pollinated from the original plant of *nanella-brevistylis* (20.76-34). The contents of 1 capsule gave 114 seedlings, including 5 stumpy, and a residue of 126 sterile seeds, a germination of 47.5 percent. This was seed fertility distinctly higher than the line of *Lamarckiana* which, tested in the years 1916, 1917, 1919 and 1924, gave germinations, respectively, of 36, 36, 39 and 37 percent. Mortality among young plants and in the field permitted only 56 plants to mature, distributed as 34 *Lamarckiana*, 21 *nanella* and 1 *scintillans*. In no plant of this F_1 was there a suggestion of *brevistylis* characters, which were therefore completely recessive to the long-style complex.

The splitting of this F_1 generation into the two phenotypes, *Lamarckiana* and *nanella*, follows the same system as when crosses between *Lamarckiana* and *nanella* give in the F_1 large proportions of *nanella* plants among the *Lamarckiana* (DE VRIES 1913, p. 291), a performance which clearly indicates that genes for *nanella* are present in the heterozygous *Lamarckiana*. In this case, however, the *Lamarckiana* and *nanella*-like plants carry the recessive gene for the short style complex, as is proved by their performance in the F_2 generation.

Of this culture 4 plants *nanella*-like and 6 plants *Lamarckiana*-like were selfed, and their progeny in the F_2 will now be considered.

THE F_2 GENERATIONS FROM *Lamarckiana*×*nanella-brevistylis*

Culture 23.51 was from an F_1 *Lamarckiana*-like plant (21.30-1). The contents of 2 capsules gave 137 seedlings, of which 35 were stumpy, presenting at once a high mortality. There were 338 sterile seeds, a

TABLE 1

The F_2 generations from Lamarckiana × nanella-brevistylis.

CULTURE	APPEARANCE OF F1 PARENT PLANT	SEEDLINGS	STERILE SEEDS	PERCENTAGE OF GERMINATION	*brevistylis*	*Lamarckiana*	*nanella-brevistylis*	*nanella*	OTHER FORMS	FAILED TO MATURE	STUMPY SEEDLINGS	DIED INCLUDING ALL STUMPY SEEDLINGS	RATIO OF SHORT-STYLED TO LONG-STYLED PLANTS
23.51	21.30-1 *Lamarckiana*	137 (2 capsules)	338	28.8	17	58	.	.	1 *oblonga* 2 *nanella*	1 *scintillans*-like rosette 8 *Lamarckiana*-like rosettes	35	50	1 : 3.6
23.52	21.30-2 *nanella*	86 (2 capsules)	172	33.3	.		24	44			4	18	1 : 1.8
23.53	21.30-3 *Lamarckiana*	119 (1 capsule)	206	36.6	15	81			1 hero (short-styled) 1 *nanella*	4 *Lamarckiana*-like rosettes	9	17	1 : 5.1
23.54	21.30-4 *nanella*	127 (1 capsule)	146	46.5			18	77			11	32	1 : 4.3
23.55	21.30-5 *nanella*	96 (2 capsules)	179	34.9	..		18	63	1 narrow-leaved dwarf (short-styled)	1 *nanella*-like rosette	6	13	1 : 3.3
23.56	21.30-9 *Lamarckiana*	115 (1 capsule)	232	33.1	9	70	..				11	36	1 : 7.8
23.57	21.30-13 *Lamarckiana*	129 (1 capsule)	233	35.6	20	83	.	..		3 *scintillans*-like rosettes 2 *Lamarckiana*-like rosettes	7	21	1 : 4.1
23.58	21.30-16 *Lamarckiana*	91 (1 capsule)	171	34.7	13	51	.	.	1 *nanella-brevistylis*	1 *scintillans*-like rosette	7	25	1 : 3.6
23.59	21.30-17 *Lamarckiana*	98 (1 capsule)	164	37.4	13	68	..	..	1 *nanella*	2 *scintillans*-like rosettes 1 *Lamarckiana*-like rosette	5	13	1 : 5.3
23.60	21.30-94 *nanella*	138 (2 capsules)	273	33.6	..	..	20	60		2 narrow-leaved dwarf rosettes 1 broad-leaved dwarf rosette	18	55	1 : 3

germination of 28.8 percent. Further mortality and the fact that 8 plants remained as rosettes reduced the number of plants that reached maturity to 78, distributed as 17 *brevistylis*, 58 *Lamarckiana*, 2 *nanella*, and 1 plant of *oblonga*. The proportion of *brevistylis* plants to all long-styled forms was then 17 to 61, a ratio of 1 : 3.6. (See table 1.)

Culture 23.52 was from an F_1 *nanella*-like plant (21.30-2). The contents of 2 capsules gave 86 seedlings, of which 4 were stumpy. There was a residue of 172 sterile seeds, a germination of 33.3 percent. Mortality reduced the culture at maturity to 68 plants, of which 24 were *nanella-brevistylis* and 44 *nanella*, a ratio of 1 : 1.8.

Culture 23.53 was from an F_1 *Lamarckiana*-like plant (21.30-3), one capsule of which gave 119 seedlings, including 9 stumpy, and a residue of 206 sterile seeds, a germination of 36.6 percent. There matured 98 plants distributed as 15 *brevistylis*, 1 robust short-styled plant with habit and foliage *gigas*-like, 81 *Lamarckiana* and 1 *nanella*. The short-styled plants were then as 16 to 82, a ratio of 1 : 5.1. The presence of the hero plant in this culture is a matter of interest. Its pollen was abundant but mostly shriveled. Unfortunately, the plant flowered too late in the season to make possible successful pollinations.

Culture 23.54 was from an F_1 *nanella*-like plant (21.30-4), one capsule of which gave 127 seedlings, including 11 stumpy, and 146 sterile seeds, a germination of 46.5 percent. Of the 95 plants which matured, 18 were *nanella-brevistylis* and 77 *nanella*, a ratio of 1 : 4.3.

Culture 23.55 was from an F_1 *nanella*-like plant (21.30-5). The contents of 2 medium-sized capsules gave 96 seedlings, including 6 stumpy, and 179 sterile seeds, a germination of 34.9 percent. There matured 82 plants distributed as 18 *nanella-brevistylis*, 1 narrow-leaved dwarf, short styled plant, and 63 *nanella*. The proportion of short styled plants to *nanella* was as 19 to 63, a ratio of 1 : 3.3.

Culture 23.56 was from an F_1 *Lamarckiana*-like plant (21.30-9), one capsule of which gave 115 seedlings, including 11 stumpy, and 232 sterile seeds, a germination of 33.1 percent. Heavy mortality reduced the number of plants that reached maturity to 79, of which 9 were *brevistylis* and 70 *Lamarckiana*, a ratio of 1 : 7.8.

Culture 23.57 was from an F_1 *Lamarckiana*-like plant (21.30-13). The contents of 1 capsule gave 129 seedlings, including 7 stumpy, and 233 sterile seeds, a germination of 35.6 percent. There remained in the field 3 rosettes *scintillans*-like and 2 rosettes *Lamarckiana*-like. There matured 103 plants of which 20 were *brevistylis* and 83 *Lamarckiana*, a ratio of 1 : 4.1.

Culture 23.58 was from an F_1 *Lamarckiana*-like plant (21.30-16) one capsule of which gave 91 seedlings, including 7 stumpy, and 171 sterile seeds, a germination of 34.7 percent. A *scintillans*-like rosette remained in the field. There matured 13 plants of *brevistylis*, 1 plant of *nanella-brevistylis* and 51 plants of *Lamarckiana*. The proportion of short-styled plants to *Lamarckiana* was then as 14 to 51, a ratio of 1 : 3.6. Of especial interest was the plant of *nanella-brevistylis*, giving another instance of *brevistylis* characters passing over to a *nanella* plant from an immediate *Lamarckiana*-like parent heterozygous for *brevistylis* characters. This seems to be a case parallel to the appearance of the original plant of *nanella-brevistylis* (20.76-34) which came out in an F_2 from the cross *brevistylis* × *Lamarckiana*.

Culture 23.59 was from an F_1 *Lamarckiana*-like plant (21.30-17) one capsule of which gave 98 seedlings, including 5 stumpy, and 164 sterile seeds, a germination of 37.4 percent. There remained as rosettes 2 *scintillans*-like and 1 *Lamarckiana*-like. There came to maturity 83 plants distributed as 13 *brevistylis*, 68 *Lamarckiana* and 1 *nanella*. The ratio of *brevistylis* to long-styled plants was as 13 to 69, or 1 : 5.3.

Culture 23.60 was from an F_1 *nanella*-like plant (21.30-94). The contents of 2 medium-sized capsules gave 138 seedlings, including 18 stumpy, and 273 sterile seeds, a germination of 33.6 percent. There remained in the field 2 narrow-leaved dwarf rosettes and 1 broad-leaved dwarf rosette. Heavy mortality reduced the number of plants that matured to 80, distributed as 20 *nanella-brevistylis* and 60 *nanella*, a ratio of 1 : 3.

The results from the ten cultures of the F_2 generations from *Lamarckiana* × *nanella-brevistylis*, as given above, are presented in table 1. Certain general conclusions stand out clearly. The segregation of short-styled plants from long-styled was absolutely clear, there being no intergrades for any of the characters that make up the short-style complex. The proportion of short styles to long was very close to the monohybrid ratio of 1 : 3 in four of the cultures (23.51, 55,58,60), ranging from 1 : 3 to 1 : 3.6. In five cultures the ratios ranged from 1 : 4.1 to 1 : 7.8, thus presenting smaller proportions of short-styled plants than the ratio 1 : 3. In one culture (23.52) the ratio of 1 : 1.8 showed a larger proportion of short-styled plants than was to be expected. Thus, in general, these results agreed with those of my earlier studies in that there appear to be factors working against the full numerical expression of the short-styled segregates.

As was to be expected, the F_2 cultures from the F_1 *nanella*-like plants gave only dwarf plants. The cultures from the F_1 *Lamarckiana*-like plants threw occasional variants such as *nanella*, *oblonga*, a hero type with short styles, *scintillans*-like rosettes, and a narrow-leaved short-styled dwarf. In one culture (23.58) a *nanella-brevistylis* again appeared from an F_1 *Lamarckiana*-like parent heterozygous for *brevistylis* characters.

The class of stumpy seedlings appeared in every culture and sometimes in large numbers and materially raised the totals of plants that died during the season. Stumpy seedlings, since they develop no roots, live only a few days. The other mortality, as usual, was distributed throughout the season, but most largely affected plants in earlier stages of development.

COMPARATIVE GROWTH RATES OF POLLEN TUBES CARRYING FACTORS FOR SHORT AND LONG STYLES, RESPECTIVELY

Following the method employed by HERIBERT-NILSSON (1920), a test was made of the comparative growth rates of pollen tubes carrying genes for short and long styles, respectively. The procedure was to cut off the styles of flowers from an F_1 plant, close to the ovaries, at various time intervals following pollination. If pollen tubes carrying genes for short styles should grow more slowly than those with genes for long styles it should be possible to obtain ratios in the F_2 with larger proportions of long-styled plants than usual if the operation was performed at a time before many short-styled pollen tubes had reached the ovary. The plant selected for the experiment was the *Lamarckiana*-like F_1 plant 20.30-1, *Lamarckiana*×*nanella-brevistylis*, normally developed capsules of which gave culture 23.51 in table 1.

As shown in table 2, no fertilizations were effected until 22 hours after pollination, although attempts were made to obtain seed 17, 18, 19, 20 and 21 hours after pollination. Four attempts to obtain seed after 22 hours were failures, but 7 small capsules about 12 mm long were obtained from other trials. Larger capsules, 14 mm long, were produced 23 hours after pollination, and still larger, 15 to 16 mm long, when the interval between pollination and the cutting of the style was 24 hours. The ratio of short to long styles following a normal withering of the flowers on this plant was given by culture 23.51. The results of the experiment were as follows.

Culture 23.81 was from 7 capsules, 12 mm long, developed 22 hours after pollination. The harvest gave 54 seedlings, including 5 stumpy, and there was a residue of 204 sterile seeds, a germination of 20.9 percent. An *oblonga*-like rosette, a *scintillans*-like rosette and a *Lamarckiana*-like rosette remained in the field. There matured 5 plants of *brevistylis* and 25 plants of *Lamarckiana*, giving proportions of short-styled to long-styled in the ratio of 1 : 5.

Culture 23.82 was from 3 capsules, 14 mm long, developed 23 hours after pollination. There were 68 seedlings, including 4 stumpy, and 238 sterile seeds, a germination of 22.2 percent. There remained in the field 2 *Lamarckiana*-like rosettes. There matured 8 plants of *brevistylis* and 40 plants of *Lamarckiana*, a ratio of 1 : 5.

Culture 23.83 was from 4 capsules, 15 to 16 mm long, developed 24 hours after pollination. These gave 101 seedlings, including 7 stumpy, and 406 sterile seeds, a germination of 19.9 percent. There remained in the field 2 *oblonga*-like rosettes and 3 *Lamarckiana*-like. There matured 20 plants of *brevistylis*, 1 plant of *nanella-brevistylis*, 58 plants of *Lamarckiana* and 1 plant of *nanella*. The proportion of short-styled plants to long-styled was then as 21 to 59, a ratio of 1 : 2.8. We note again, as in culture 23.58, table 1, the appearance of a *nanella-brevistylis* from an F_1 *Lamarckiana*-like plant heterozygous for *brevistylis* characters.

For purpose of comparison with the results of the experiments from cultures 23.81, 23.82 and 23.83, there is given at the bottom of table 2 the data from culture 23.51 repeated from table 1. Culture 23.51 was from seeds of the same plant, 20.30-1, on which the experiments were performed, but its flowers withered normally and consequently the pollen tubes had the full time to accomplish all fertilizations possible.

A comparison of the four cultures (see table 2) offers the following conclusions. (1) With increasing length of time for the growth of pollen tubes there are produced higher average numbers of seeds per capsule (27, 102, 127 and 237 seeds), and a corresponding increase in the size of the capsules (12, 14, 15, 16 and 21 mm). (2) The percentages of viable seeds are lower when full fertilization is prevented (20.9, 22.2, 19.9 and 28.8 percents of germination). This is probably due to less efficient nutrition of the smaller capsules. (3) The ratio of short-styled to long-styled plants in the F_2 was lower (1 : 5) when the time interval for the growth of pollen tubes was 22 and 23 hours. The ratio (1 : 2.8) was close to expectations when the time interval was 24 hours, agreeing closely with the ratio (1 : 3.6) from capsules following a normal withering of the flowers.

Although the data are not extensive, nevertheless they suggest that pollen tubes carrying the genes for the short-style complex do not grow as rapidly as those with genes for the long-style complex. Long-styled pollen tubes apparently reach the ovary earlier and may be expected to effect the fertilization of a larger proportion of the ovules than would otherwise take place, a result expressed by lower proportions of *brevistylis* segregates. The difference in rate of growth is not great, since a time inter-

TABLE 2

The F_2 generations after various time intervals permitted for the growth of pollen tubes down the style. Experiments with the F_1 Lamarckiana-like plant, 21.30–1, of the cross Lamarckiana×nanella-brevistylis. Styles were cut close to the ovaries after the time intervals following pollination as given in the first column.

TIME INTERVAL FOR GROWTH OF POLLEN TUBES	NUMBER OF FLOWERS POLLINATED	NUMBER OF CAPSULES DEVELOPED	LENGTH OF CAPSULE	AVERAGE NUMBER OF SEEDS	CULTURE	SEEDLINGS	STERILE SEEDS	PERCENTAGE OF GERMINATION	*brevistylis*	*Lamarckiana*	OTHER FORMS	FAILED TO MATURE	STUMPY SEEDLINGS	DIED INCLUDING ALL STUMPY SEEDLINGS	RATIO OF SHORT-STYLED TO LONG-STYLED PLANTS
17 hours	3	none													
18 hours	4	none													
19 hours	6	none													
20 hours	5	none													
21 hours	3	none													
22 hours	4	none													
22 hours	7	7	12 mm	37	23.81	54	204	20.9	5	25		1 *oblonga*-like rosette 1 *scintillans*-like rosette 1 *Lamarckiana*-like rosette	5	21	1 : 5
23 hours	3	3	14 mm	102	23.82	68	238	22.2	8	40		2 *Lamarckiana*-like rosettes	4	18	1 : 5
24 hours	4	4	15 to 16	127	23.83	101	406	19.9	20	58	1 *nanella-brevistylis* 1 *nanella*	2 *oblonga*-like rosettes 3 *Lamarckiana*-like rosettes	7	16	1 : 2.8
A normal withering of the flower	2	2	21 mm	237	23.51	137	338	28.8	17	58	1 *oblonga* 2 *nanella*	1 *scintillans*-like 8 *Lamarckiana*-like rosettes	35	50	1 : 3.6

val of 24 hours gives the short-styled pollen tubes an opportunity to record their presence by a ratio approximating the monohybrid 1 : 3.

It is admitted that conclusions of this character must be taken with due allowance for the difficulties of experimentation. The principal weakness lies in the loss of plants through death, including the large group of stumpy seedlings. We should very much like to know the genetical constitution of the stumpy seedlings. However, these results are presented as pointing towards the sort of experimental work necessary to solve some of the problems presented by seeming departures of Oenothera genetics from Mendelian principles. The writer has no sympathy with a group of Oenothera workers which has held that Oenothera genetics follows a system apart from the fundamentals of Mendelism which is an orderly process of segregation from heterozygous stock. In Oenothera material orderly segregation is present but usually in complex relations for specific reasons which research should endeavor to determine.

DISCUSSION

My earlier studies (DAVIS 1918, 1921) showed that malnutrition of developing seeds on F_1 plants in crosses between *brevistylis* and *Lamarckiana* will kill a larger proportion of *brevistylis* zygotes or embryos than of *Lamarckiana.* As a result *brevistylis* segregates in the F_2 and in back-crosses of the F_1 to the *brevistylis* parent will be in smaller proportions than the expected 1 : 3 and 1 : 1 ratios. The experiments here recorded on rate of pollen-tube growth indicate that pollen tubes with genes for short styles grow more slowly than do those with genes for long styles. Thus, we seem to have discovered two factors which work against the full expression of *brevistylis* segregates from crosses involving plants heterozygous for *brevistylis* characters. The facts of segregation are unmistakable; the ratios clearly indicate the Mendelian monohybrid system; the departures from expectations are due to factors which operate to the disadvantage of *brevistylis* segregates.

Some geneticists may be tempted to interpret these cases as tied up with lethals, but I can see no justification for such considerations. The death of zygotes or embryos of *brevistylis* in competition with those of *Lamarckiana* under conditions of limited nutrition is a phase in the struggle for existence. The apparently slower growth of pollen tubes carrying genes for *brevistylis* is a matter of physiology, but it is serious to the species in that it must lead to lower proportions of *brevistylis* fertilizations when there are present a larger number of pollen tubes with genes for long styles than there are ovules to be fertilized.

What may be the interpretation of the facts of dwarf and of short-style segregation from the crosses here discussed? We have the following basis on which to build genetical formulae: (1) *Brevistylis* characters are recessive to those of *Lamarckiana*, and short styles when present in *nanella* (*nanella-brevistylis*) are recessive to typical *nanella*. (2) *Nanella* breeds true, but *Lamarckiana* throws its small percentage of *nanella* plants. (3) *Lamarckiana*×*brevistylis* gives about the same proportion of *nanella* as *Lamarckiana* selfed (DE VRIES 1901), showing that *brevistylis* is also heterozygous for tall stature. (4) *Nanella* crossed to a number of Oenothera species (DE VRIES 1913, p. 292) gives no dwarfs in the F_1, but dwarfs appear in the F_2, which indicates that dwarfness is recessive. (5) *Nanella* crossed to *Lamarckiana* gives *nanella* in fair proportions with *Lamarckiana* (DE VRIES 1913, p. 207), which implies that *Lamarckiana* carries *nanella* genes as recessives. (6) *Nanella* pollinated by *brevistylis* is reported (DE VRIES 1913, p. 207) to give *nanella* in proportions of 31 to 50 percent, again indicating that *brevistylis* also carries *nanella* genes as recessives. The *brevistylis* characters do not appear in the F_1 of this cross (DE VRIES 1913, p. 190) but come out in the F_2. (7) The small percentages of *nanella* from selfed *Lamarckiana* may be due to conditions which permit only an occasional successful union of gametes carrying the recessive genes to form zygotes homozygous for dwarfness, or which permit only occasionally the survival and development of such zygotes. There are sufficient degrees of sterility, both gametic and zygotic, to permit of either possibility.

Let *T* represent the gene for the stature of *Lamarckiana* and *brevistylis* and *t* its allelomorph for the recessive dwarfness of *nanella*. Let *L* stand for the long style of *Lamarckiana* and *nanella* and *l* for the recessive short style of *brevistlyis* and *nanella-brevistylis*. Then, if the above data are correct, and if the genes for stature and for style length are in different chromosomes, we may assume the formula of *Lamarckiana* to be *TtLL*, *brevistylis TtII*, *nanella ttLL*, and *nanella-brevistylis ttll*. Should the genes for stature and style length be in the same chromosome, the formula for *Lamarckiana* would be *TLtL*, *brevistylis Tltl*, *nanella tLtL*, and *nanella-brevistylis tltl*.

There are then two sets of hypothetical genetical formulae which must be tested in their application to the breeding data presented in this paper. The first set places the genes for stature and for style length in different chromosomes; the second set assumes that these genes are linked in the same chromosome. The origin of the first plant of *nanella-brevistylis* in the F_2 of the cross *brevistylis*×*Lamarckiana* is permitted by either set of formulae. Both sets of formulae allow the segregation of the short-style complex in the F_2 generations from the cross *nanella*×*nanella-brevistylis* and from the F_1 *nanella*-like plants of the cross *Lamarckiana*×*nanella-brevistylis*. The test of the merits of the two sets of genetical formulae comes with their application to the breeding behavior of the F_1 *Lamarckiana*-like plants from the cross *Lamarckiana*×*nanella-brevistylis*.

By the second set of formulae (assuming linkage of the genes for stature and style length in the same chromosome) the cross *Lamarckiana* (*TLtL*) ×*nanella-brevistylis* (*tltl*) would give in the F_1 *Lamarckiana*-like plants (*TLtl*) and *nanella*-like plants (*tLtl*). The *Lamarckiana*-like F_1 plants (*TLtl*), selfed, should by this hypothesis segregate these genes as 1 *TLTL* : 2 *TLtl* : 1 *tltl*, which would be 3 *Lamarckiana*-like plants to 1 *nanella-brevistylis*. The facts are that such plants (tables 1 and 2) give *brevistylis* and *Lamarckiana* in ratios close to 1 : 3, together with occasional dwarfs.

By the first set of formulae (assuming the genes for stature and style length to lie in different chromosomes) the cross *Lamarckiana* (*TtLL*) ×*nanella-brevistylis* (*ttll*) would give in the F_1 *Lamarckiana*-like plants (*TtLl*) and *nanella*-like plants (*ttLl*). The *Lamarckiana*-like plants (*TtLl*) being dihybrids would give, if segregation were fully expressed: 1 *TTLL*, 2 *TTLl*, 2 *TtLL*, 4 *TtLl*, 1 *TTll*, 2 *Ttll*, 1 *ttLL*, 2 *ttLl*, 1 *ttll*. This is a distribution of 9 *Lamarckiana*-like : 3 *brevistylis*-like : 3 *nanella*-like : 1 *nanella-brevistylis*. These segregates all appear, as is shown from cultures 23.51-53-56-57-58-59 of table 1, and cultures 23.81-82-83 and 23.51 (repeated) of table 2, which thus satisfy the formulae that assume genes for stature and style length to lie in different chromosomes.

Thus, expectations are realized as to the character of the segregates from the *Lamarckiana*-like plants, but there is the same deficiency of dwarfs characteristic of the progeny from *Lamarckiana*. The total of dwarfs (*nanella* and *nanella-brevistylis*) from cultures 23.51-53-56-57-58-59 of table 1 is only 5 plants in contrast to 498 tall plants (*Lamarckiana* and *brevistylis*); they make up 1 percent of the cultures, which is close to the percentage of *nanella* from *Lamarckiana* as reported by DE VRIES.

Of the full set of theoretically possible segregates from the F_1 *Lamarckiana*-like plants listed above, it is possible that those homozygous for tallness (*TT*) never develop, since we know of no lines of *Lamarckiana* and *brevistylis* homozygous for tall stature. If this is so, we may take from the list the plant *TTLL*, the 2 plants *TTLl* and the plant *TTll*, and the proportions of the four types would become 6 *Lamarckiana*-like : 2 *brevistylis*-like : 3 *nanella* like : 1 *nanella-brevistylis*. The ratio of short-styled plants to long-styled would remain as before, 1 : 3, but the ratio of dwarfs to tall would be 1 : 2.

Let us apply MULLER'S (1918) hypothesis of balanced lethals to the situation presented by the small percentage of dwarf segregates from *Lamarckiana* in selfed lines and from the F_1 *Lamarckiana* plants of the cross *Lamarckiana*×*nanella-brevistylis*. We assume for the chromosomes carrying the genes for high stature, T, and for dwarfness, t, two different lethals, v_1 and v_2. The formula for *Lamarckiana* would then be Tv_1tv_2LL, for *brevistylis*, Tv_1tv_2ll, for *nanella*, tv_1tv_2LL, and for *nanella-brevistylis*, tv_1tv_2ll. If from these hypothetical formulae the segregation is worked out for *Lamarckiana* and *nanella* selfed, and from the crosses between *Lamarckiana* and *nanella*, between *Lamarckiana* and *nanella-brevistylis* and between *nanella* and *nanella-brevistylis*, it will be found that certain classes of segregates will carry v_1v_1 or v_2v_2 and others the combination v_1v_2. It is assumed that the classes homozygous for either lethal (v_1v_1 or v_2v_2) fail to develop and that only such plants live as are heterozygous for both lethals (v_1v_2). The low seed fertility of *Lamarckiana*, *brevistylis*, *nanella* and *nanella-brevistylis*,—less than 50 percent,—permits this hypothesis.

The character of the segregation of long and short styles is not affected by the assumptions involved in the hypothesis of balanced lethals. The hypothesis will, however, only permit of the appearance of dwarfs from *Lamarckiana* or from the F_1 *Lamarckiana* plants of the cross *Lamarckiana* ×*nanella-brevistylis* on the assumption of repeated and regular crossings over between the genes for stature and the lethals. Such crossing over would give dwarf plants, the ratio of which would be determined by the frequency of the crossing over. The hypothesis of balanced lethals is then concerned with a special modification of segregation to give dwarfs by crossing over and does not admit a problem of deficiency. For this reason it is much simpler than an hypothesis which allows a regular segregation of dwarfs, but must explain why they fall so very far short of the expected proportions.

I have tried to find evidence for some simple differential that would account for the small proportions of dwarfs without involving the assumption of lethals. Thus, if pollen tubes from pollen grains carrying genes for dwarfness grew more slowly than those from pollen grains with genes for tall stature there would be a physiological factor working against the appearance of dwarfs whenever there were many more pollen tubes present than ovules to be fertilized. Under such conditions the segregation from an F_1 *Lamarckiana*-like plant ($TtLl$) of the cross *Lamarckiana* ×*nanella-brevistylis* might give only the tall plants 1 $TTLL$, 2 $TTLl$, 1 *TTll*, 1 *TtLL*, 2 *TtLl*, 1 *Ttll*, with perhaps an occasional dwarf. The data assembled in table 2, however, do not help us with the problem, even though the dwarfs did not come out in cultures 23.81 and 23.82, because the data are not sufficiently extensive to justify conclusions with the factor of probable error so large. It is possible that further experimentation may give clearer evidence of physiological factors to account for a deficiency of dwarfs, but as matters stand the hypothesis of balanced lethals is likely to appeal more strongly to the geneticist.

Finally, there is the hypothesis of mutation to account for the appearance of dwarfs from *Lamarckiana*. DE VRIES believed that *Lamarckiana* is pure for tall stature, which would mean that its formula would be *TT* and not *Tt* as assumed in this paper. The mutation of *T* to *t* in sufficient frequency would result in the production by *Lamarckiana* of gametes carrying *t*, which through union with one another would give *nanella*, *tt*. Thus, *Lamarckiana* in selfed line might be expected to throw *nanella* and when crossed to *nanella* would be expected to give a progeny of *Lamarckiana*-like forms, *Tt*, and *nanella*. The hypothesis assumes mutation of a frequency far beyond that which seems probable from the experience of geneticists in such thoroughly studied material as *Drosophila* and maize. It must assume that the *Lamarckiana*-like plants (*Tt*) coming out of the crosses with *nanella* are different from the parent *Lamarckiana*, of which there is no evidence, or that *t* mutates back to *T*. The regularity of the appearance of *nanella* from *Lamarckiana* and from the crosses between *nanella* and *Lamarckiana* will seem to the geneticist as much more likely to rest upon some expression of segregation. The fact that *nanella* crossed to certain other species of Oenothera does not give dwarfs, implies that *Lamarckiana* is not homozygous for tall stature. Finally, there is the accumulated evidence from various viewpoints, too numerous to be discussed here, which throws doubt on the genetical purity of *Lamarckiana* and of most Oenotheras that have been carefully studied, and leads us to believe that the genus is chiefly an assemblage of impure species. Attempts to establish evidence for mutation from such material must expect keen scrutiny and criticism from geneticists.

SUMMARY

Oenothera nanella-brevistylis is a dwarf with the habit and stature of *nanella* but with those characters of *brevistylis* which make up the short-style complex. The short-style complex consists of a short style with deformed or rudimentary stigma lobes, bud cones more stout and with short sepal tips, broader less pointed bracts, and ovary partly superior

and ripening few or no seeds. The plant first appeared in an F_2 from the cross *brevistylis*×*Lamarckiana* (DAVIS 1921, p. 580, table 3). This plant produced no seed when pollinated, and consequently crosses could be made with it only as the pollen parent.

The F_1 generation of *nanella*×*nanella-brevistylis* consists of plants uniformly *nanella*-like. Thus, the short-style complex of *nanella-brevistylis* is recessive to the long-style complex of *nanella*, as are the same characters in *brevistylis* recessive to those of *Lamarckiana.*

F_2 generations from two selfed F_1 plants of *nanella*×*nanella-brevistylis* presented plants uniformly dwarf but segregating sharply as having either the long-style complex of *nanella* or the short-style complex of *nanella-brevistylis.* The ratios of *nanella-brevistylis* to *nanella* were 1 : 5.7 (culture 23.49) and 1 : 4.5 (culture 23.50), thus departing from the expected monohybrid ratio of 1 : 3 with a deficiency of *nanella-brevistylis* probably due to some factors working against the full expression of this segregate. This deficiency of *nanella-brevistylis* agreed with a similar deficiency of *brevistylis* segregates in the F_2 of its cross with *Lamarckiana* (DAVIS 1918, 1921).

The F_1 generation of *Lamarckiana*×*nanella-brevistylis* consisted of 30 plants *Lamarckiana*-like, 21 plants *nanella*-like and one plant *scintillans*-like. There was no evidence of the short-style complex in this generation, showing it to be completely recessive to the long-style complex.

F_2 generations from *Lamarckiana*×*nanella-brevistylis* were grown from the seed of 4 *nanella*-like and 6 *Lamarckiana*-like plants of the F_1. The F_2 generations from the selfed *nanella*-like F_1 plants (table 1) consisted of *nanella-brevistylis* and *nanella* in ratios of 1 : 1.8, 1 : 3, 1 : 3.3 and 1 : 4.3 (cultures 23.52, 60, 55, 54). The F_2 generations from the selfed *Lamarckiana*-like F_1 plants (table 1) consisted of *brevistylis* and *Lamarckiana* in ratios of 1 : 3.6, 1 : 3.6, 1 : 4.1, 1 : 5.1, 1 : 5.3 and 1 : 7.8 (cultures 23.51, 58, 57, 53, 59, 56). There was a scattering of variants in the cultures from the F_1 *Lamarckiana*-like plants (table 1) including one short-styled hero plant (culture 23.52) and one *nanella-brevistylis* (culture 23.58). The segregation of the short-style complex from the long-style was perfectly sharp; but as in my earlier studies on crosses between *brevistylis* and *Lamarckiana* (DAVIS 1918, 1921), there was generally a smaller proportion of *brevistylis* segregates than the expected 1 : 3 ratio would require.

Experiments on comparative growth rate of pollen tubes on a selected F_1 plant (21.30-1, *Lamarckiana*×*nanella-brevistylis*) carrying genes for the short- and long-style complexes showed (table 2): (1) That no fertilization was effected until about 22 hours after pollination. (2) That small capsules were developed 22 hours after pollination, the seed of which gave an F_2 generation with the proportions of *brevistylis* to *Lamarckiana* in the ratio of 1 : 5 (culture 23.81). (3) That somewhat larger capsules, developed 23 hours after pollination, gave an F_2 with *brevistylis* and *Lamarckiana* in the ratio also of 1 : 5 (culture 23.82). (4) That still larger capsules developed 24 hours after pollination gave *brevistylis* to *Lamarckiana* in the ratio of 1 : 2.8 (culture 23.83). In this culture appeared the third plant of *nanella-brevistylis*. Capsules on the same F_1 plant, developed after a normal withering of the flowers, gave an F_2 with the proportions of *brevistylis* to *Lamarckiana* in the ratio of 1 : 3.6. These data, although not extensive, suggest that pollen tubes carrying genes for the short-style complex do not grow as rapidly as do those with genes for the long-style complex. Thus a factor has perhaps been discovered which may operate to lower the proportions of short-styled segregates.

From my earlier work (DAVIS 1921) and the experiments recorded above we may have established two factors which work to the disadvantage of short-styled segregates: first, malnutrition of zygotes and embryos; and second, slower growth rate of pollen tubes carrying genes for the short-style complex. The results of such operations would be smaller proportions of short-styled segregates than theory would indicate, which seems to agree with the facts.

From the breeding data given in the discussion it is clear that the genes for stature (*T* and *t*) and for style length (*L* and *l*) are in different chromosomes as suggested by SHULL (1921). Therefore, with respect to the relation of these genes to one another the formula for *Lamarckiana* is *TtLL*, *brevistylis*, *Ttll*, *nanella*, *ttLL* and *nanella-brevistylis*, *ttll*. By these formulae the segregation of the short-style complex from this material should follow the monohybrid ratios, which agrees with the facts except that, as noted above, the proportion of short-styled segregates is somewhat smaller than expectation. The formulae also permit the appearance of the double recessive *nanella-brevistylis*, from appropriate crosses.

There is a constant and marked deficiency of dwarfs from *Lamarckiana*, from *brevistylis*, and from crosses involving these two types. In the absence of evidence for some differential working against the appearance of dwarfs, MULLER'S hypothesis of balanced lethals offers the most satisfactory interpretation for this breeding behavior.

Let v_1 and v_2 stand for two lethals in the chromosomes concerned with stature, and the formulae become for *Lamarckiana*, Tv_1tv_2LL, for *brevistylis*, Tv_1tv_2ll, for *nanella*, tv_1tv_2LL, and for *nanella-brevistylis*, tv_1tv_2ll. Crosses involving these formulae will give classes of segregates carrying v_1v_1 or v_2v_2, and others with the combination v_1v_2. It is assumed that the classes homozygous for either lethal (v_1v_1 or v_2v_2) fail to develop, and that only such plants live through the fertile seeds as are heterozygous for both lethals (v_1v_2). The proportions of fertile seeds,—under 50 percent,—in *Lamarckiana*, *brevistylis*, *nanella* and *nanella-brevistylis*, permit this assumption of zygotic lethals. On the further assumption of repeated and regular crossings over between the genes for stature and the lethals, dwarfs would appear in proportions determined by the crossover frequency. The regularity of the segregation of the short-styled complex in a different chromosome is not affected by these lethals associated with the chromosome carrying genes for stature.

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THE PRECIPITIN REACTION IN RELATION TO GRAFTING[1]

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INTRODUCTION

Since it is apparent that one of the principal factors for the successful production of grafts, and possibly also for hybridization, is that the plants should be nearly related, and since the relationship among animals has been successfully studied by means of immunity reactions, I have tried to employ these more exact and scientific methods in the investigation of plants. For this purpose, I have used what is known as the "precipitin reaction."

This reaction, discovered by KRAUS in 1897, gave to UHLENHUTH (1900, 1901), WASSERMANN (1903) and NUTTALL (1904) the means of differentiating the various species of animals through their specific proteins. As precipitins are formed with any soluble animal or vegetable protein, they were made use of also in botany. The phytoprecipitins, sa they are called inbotany, were first studied by KOWARSKY (1901), MAGNUS and FRIEDENTHAL (1906), MAGNUS (1908), GASIS (1908) and others. These authors proved that specific precipitins could be obtained from various plants, but did not attempt a methodical investigation in this field, as had been done with animals.

In 1913 the Botanical School of Königsberg, under the direction of MEZ, started methodical researches for the classification of plants by

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means of the precipitins. This school, guided by the previous classifications based on the morphological and structural appearances of plants, started to test them by means of this immunological reaction (GOHLKE 1913), and, although the new classification is still in its infancy, they have already examined a large number of plants. MEZ and GOHLKE (1913) have studied the Angiosperms; MEZ and LANGE (1914) the Ranales; MEZ and PREUSS (1914) the Parietales; MEZ and KIRSTEIN (1920) the Gymnosperms; SALTZMANN (1924) has reviewed once more the Ranales and completed the Gnetales; RAEDER (1924) the Adoxaceae, Loranthaceae, Ericaceae, Polygalaceae, Empetraceae and Hypericaceae; GUTTMANN (1924) the Archegoniatae; STEINECKE (1925) the genealogy of the Algae. This work, of the most interesting nature on account of its fundamental character, is not yet generally acknowledged, but, as RAEDER says, "the genealogy of plants established by means of the sero-diagnostic reactions is gaining more and more recognition."

As the literature seems to be mute on the subject of immunological reaction in relation to grafting among plants, I undertook a study of the behavior of the precipitin reaction in relation to a few plants known to be successfully intergrafted. The external characteristics are not an absolutely reliable rule by which one can judge the degree of the internal and constitutional relationship either for the vegetative or for the sexual affinity between the different species.

The object of this research was to investigate whether plants having been classified in families by their morphological characteristics and generally known to be capable of being intergrafted, would react in the same way to the specific biological reaction of the precipitins.

The plants which I have particularly studied are the following:

(1) Family of the Rutaceae; Genus: Citrus.
    Citrus aurantium (orange).
    Citrus limonum (lemon).
    Citrus vulgaris (bitter orange).
    Citrus decumana (grapefruit).
    Citrus nobilis (mandarin orange of China).

(2) Family of the Rosaceae; Sub-family: Pomoideae.
    Pyrus communis (pear).
    Pyrus malus (apple).

(3) Family of the Rosaceae; Sub-family: Prunoideae.
    Prunus domestica (plum).
    Prunus armeniaca (apricot).
    Prunus persica (peach).
    Prunus amygdalus (almond).

(4) Family of the Solanaceae.
    Solanum tuberosum (potato).
    Lycopersicum esculentum (tomato).
(5) Family of the Chenopodiaceae.
    Beta vulgaris (beet).
(6) Family of the Polygonaceae.
    Rheum officinale (rhubarb).
(7) Family of the Oleaceae.
    Olea europaea (olive).

The last three plants mentioned were used principally as controls.

TECHNIQUE

Preparation of the extract

The extract may be prepared from any part of the plant under investigation. I prepared all the extracts from the seeds, which, as previously noted by others, owing to the small amount of water and the large amount of soluble albumins contained in them, give an all-round greater uniformity of the extracts. The dried seeds, preferably of the same season, are reduced to a fine powder by means of a coffee grinder or, if the seeds are too large, by means of a chopping machine. The powder is freed from chaff by sifting and 5 grams of the fine flour-like powder is put into a perfectly clean and sterile Erlenmeyer flask with 50 cc of sterile 0.9 percent NaCl solution. The mixture is kept at room temperature for 12 hours and is occasionally vigorously shaken. It is then poured onto 6 layers of sterile gauze and well pressed. The milky filtrate thus obtained is then filtered twice through filter paper, Schleicher and Schüll No. 595. A sample of the clear filtrate is now taken and its albumin content is estimated by means of an Esbach albuminometer, using the Tsuchiya reagent (phosphotungstic acid 1.50 gm; hydrochloric acid 5 cc; alcohol 95 percent, 95 cc). The reading was taken after 2 hours and the amount of albumin per liter noted. As a rule, the extracts thus prepared averaged an albumin content of 3 grams per liter. As I intended to work with extracts containing approximately the same amount of albumin, if the extract did not conform to that definite amount of albumin, a correction was necessary. This correction was made, naturally, either by increasing the amount of seed flour or by diluting the extract proportionally. I laid great stress on this special point of technique in my research. The main portion of the filtrate was incubated at 60° C for one hour, after which time, it was transferred to another incubator at 37°C for 24 hours. This fractional sterilization was repeated for 3 consecutive days. Afterwards, the extracts were preserved in the ice-chest until required for use. No chemicals were used as preservatives. As a rule, all extracts thus prepared and treated showed a certain amount of precipitate due, probably, to the temperature and the acid reaction of the fluid. Although this coincidence probably diminishes the contents of active substances, it does not seem to interfere with the efficacy of the extract. Extracts which, notwithstanding all the precautions taken, gave rise to moulds, were discarded.

SALTZMANN (1924) prepared the extracts from dried and powdered leaves and to test the presence of albumin in the extracts he used GUTTERMANN'S method of demonstrating the presence of organic substances, using the Berlin bleu reaction for nitrogen and the nitroprussiate reaction for detecting the presence of sulphur. PREUSS and SALTZMANN have had good results in preparing the extract, the former with 0.1 percent and the latter with 0.5 percent NaOH.

Immunization

The animal used for immunization was the rabbit. Brown male rabbits of an average weight of 3000 grams were chosen. Before and during the process of immunization, they were fed only on carrots.

A precise neutralization of the extract previous to injecting the animal to be immunized is of capital importance. I used sodium hydroxide 0.1 percent and acetic acid 0.1 percent, using phenolphtalein as indicator.

With all the precautions of asepsis, 10 cc of carefully neutralized extract are injected into the peritoneum of the animal. The intravenous way may also be used but the intraperitoneal is safer, as, given the slow absorption of the material, it causes less reaction. The latter way is imperative, however, when immunizing animals with extracts prepared from seeds containing large amounts of toxic substances like solanin, nicotin, etc., in which case the process of immunization must be carried out with very great care and appropriate dosage. An interval of 4 days elapses between successive injections. The number of injections given averages from 7 to 10. A total quantity of 120 to 140 cc is injected, the first and last 2 injections being of 20 cc each and the intervening injections of 10 cc each. Rabbits, as a rule, bear the injections very well. Although at the beginning of the immunization they show no loss in weight, a loss is noticeable in the last few weeks of immunization.

A preliminary test of the serum of the animal so treated, when made to react with the extract used for immunization diluted in convenient proportions, will show, by the formation of a well-defined precipitate, the titer of the serum. The highest titer of my immune sera, as a rule, was between 6400 and 12,800. If the immune serum is found to give rise to a satisfactory precipitate, the animal is starved for 24 hours and then sacrificed by collecting the blood from one of the carotids, with all the precautions of asepsis, in a sterile Erlenmeyer. The blood is preserved in the ice-chest until the day after, when it is centrifuged; the supernatant clear serum is pipetted off and transferred to 5 cc glass ampoules, sealed at the flame without adding any preservatives and put in a dark, cool place.

The precipitin reaction

Both in performing the test and in preparing the reagents, I was guided by the details of technique given by UHLENHUTH, NUTTALL and the school of MEZ, and followed scrupulously those which I learned personally from WASSERMANN. In my researches, I have adopted the method of adding a constant amount of immune serum to a series of progressively diminishing dilutions of precipitinogen.

A series of 12 test tubes (7 cm in length and 1 cm in diameter) is laid in a row in a rack. The first 10 tubes are marked progressively from 50 to 25,600, meaning dilutions from 1 in 50 to 1 in 25,600. The eleventh is marked "E" for extract; the twelfth, which contains a solution of 0.9 percent sodium chloride, is marked NaCl. A second series of tubes, marked exactly like the first, is put in the second row of the rack. This second series represents the control. In tubes No. 2 to No. 11 of both series 0.5 cc of absolutely clear filtered and sterile 0.9 percent NaCl solution is put. With a sterile 1 cc pipette, 0.5 cc of very carefully neutralized and absolutely clear extract is put in tubes No. 1 and No. 11. Another 0.5 cc of the same extract is put in tube No. 2 of the front row of tubes. From tube No. 2, which now contains 1 cc of fluid, after well mixing the extract with the NaCl by sucking and expelling the fluid with the pipette, 0.5 cc is aspirated and transferred to tube No. 3. The same procedure is repeated up to tube No. 10, from which the extra 0.5 cc of the fluid is discarded. Thus, each tube in the first row now contains 0.5 cc of fluid as specified above. In the second row of tubes, the same dilutions of precipitinogen are repeated in exactly the same way as in the first. To each tube of the first row, with the exception of No. 11, is added 0.1 cc of the immune serum prepared against the homologous extract, by letting the fluid run very slowly along the wall of the test tube. To each tube of the second row 0.1 cc of fresh normal rabbit serum is added in the same way. This control shows at once whether the precipitation obtained is a specific one or whether it is due to other causes, principally the acidity of the extract. Control No. 11 of the first and second rows will show that the extract by itself does not give rise to precipitation and control No. 12 of the first row will show that normal rabbit serum plus NaCl is also absolutely free of any precipitate. With a large number of controls, the security of the results is warranted. The test tubes are now put into an incubator at 37°C. Before the reading of the reaction, in order to avoid autosuggestion on the part of the examiner, the identification marks on the test tubes are carefully concealed. A reading is taken every hour for the first 6 hours and then after 12 hours. As a rule, when the immune serum has a high titer, soon after the addition of the immune serum, a slight turbidity is noticeable. This gradually increases and, after a few minutes, gives rise to fine precipitates which are very manifest already after 15 minutes. Naturally, if the reaction is a negative one, precipitins are not formed and the fluid in the test tubes remains absolutely clear even after 24 hours. At times, slight turbidities may be noticed, which are to be interpreted as negative or false reactions, since a definite precipitin reaction is characterized by the presence of fine, minute, whitish particles, like floccules, suspended in an absolutely clear fluid. These can be seen best through a magnifying lens by holding the test tube against a black ground and comparing it with the control tubes.

Conglutination reaction

The school of MEZ has adopted a modification to the precipitin reaction, which is called "conglutination." If to the dilution of precipitinogen and its immune serum, as used with the precipitin test, a small and constant amount of ox serum be added, owing to the property of the "conglutinins" contained in the fresh active ox serum, the precipitation of the extract with its immune serum is more evident and the time of the reading of this reaction is shortened from 24 to 2.5 hours. The following is the technique used by them: One cubic centimeter of the extract of a uniform concentration is put into each of 5 small test tubes. To the first tube is added 0.08 cc of the immune serum; to the second, 0.02 cc; to the third, 0.01 cc; and to the fourth, 0.005 cc. The tubes are then incubated for 2 hours at 37°C and after this incubation each tube receives 0.4 cc of fresh ox serum. The tubes are again placed in the incubator and a reading taken every 20 minutes for 150 minutes.

Although MEZ and GOHLKE admit that the conglutination is "more complicated than the precipitation," yet they do not think that the addition of ox serum in the conglutination reaction has ever been the cause of any disturbance in the results. In fact, they have carried out all the sero-diagnostic researches in plants with the conglutination method as the principal reaction, using the precipitin reaction as a control to the first.

As the precipitin phenomenon represents a specific reaction by itself, I endeavored to adhere to the standardized rules of technique of this reaction. The principle adopted in my research, of working with antigen containing large and determined amounts of albumin, has always given me very active immune sera. Although I adopted the precipitin reaction as the main means of investigation in my research, on several occasions I tried the conglutination method as advocated by the school of MEZ. In fact, I used the sera of both oxen and sheep and, although I must avow that a certain improvement in the reaction is noticeable in the way of reading, yet, I do not agree, after all, that the slight improvement in the reaction can justify the introduction of another factor, like an extra animal serum with all its unknown sources of possible errors, which might leave the validity of the results obtained open to criticism.

RUEHLE (1924), in a publication regarding the relationship of the members of the subfamily Prunoideae, made an anatomical study of the seeds, concluding that the development in the position of the seeds (Samenlagen) of the *Prunus domestica* and the *Prunus armeniaca* is the same and that the relationship between the Pomaceae and the Prunoideae is confirmed by the presence of the obturator, the nucellar and the chalazal haustoria.

Although in my researches, with the precipitin reaction, it was easy to obtain a positive result between the various members of the Prunus subfamily, I did not succeed in obtaining a similar result between the Prunoideae and the Pomaceae. The absence of a precipitin reaction between these two subfamilies seems to coincide with the difficulty encountered in the intergrafting of these two subdivisions of the same family. The explanation of this mysterious phenomenon among plants, which, by their anatomical and other morphological characteristics would appear to have had a common origin and development, but whose common origin is not confirmed by immunological reaction, is probably to be sought in the occurrence of stereoisomeric forms of proteins and carbohydrates, as REICHERT (1916) propounds in the following quotation:

> ". . . . each kind of substance may exist in a number of forms, all of which forms have the same molecular formula and the same fundamental properties in common, but each in accordance with variations in intramolecular configuration has certain individualities which distinguish it from others. There are many known substances that exist in stereoisomeric forms and it has been found that the number of possible forms of each substance is dependent upon

> the possible number of variations of the arrangements of the molecular components in the three dimensions of space, or, in other words, of variations of molecular configuration, the possible number in case of each substance being capable of mathematical determination. Thus, we find that serum-albumin may exist in as many as a thousand million forms. Haemoglobin, the red coloring matter of vertebrate blood, is a far more complex carbon compound than serum-albumin, and theoretically may exist in forms whose number is beyond human conception, running into millions of millions. The same is true of starch."

In order to investigate more deeply this very important subject, a large series of grafting experiments ought to be carried out with plants giving the same precipitin reaction, and especially to try to solve the problem why it is more feasible to graft the quince than the apple on the pear, researches which unfortunately I did not have an opportunity of pursuing. With a methodical serological aid I think a more definite knowledge of this curious phenomenon could be obtained. As ZIEGENSPECK (1925) says:

> "The serological system throws light over much which up to the present time was problematic and gives us good prospects for further investigation into the history of our vegetation."

RESULTS AND CONCLUSIONS

The results of my studies are tabulated in table 1 from which the following conclusions seem to be justified:

(1) Plants of the genus Citrus, known to be easily intergrafted, gave a uniform positive precipitin reaction with an immune serum obtained from one of them.

(2) The subfamily of the Prunoideae, also known to intergraft successfully among themselves, although giving a uniform positive precipitin reaction among members of the same subfamily, gave a negative result when made to react with members of the subfamily Pomoideae. The Pomoideae seem to be an independent entity from the Prunoideae.

(3) The specimens of the Solanaceae examined, also known to intergraft, gave also a positive precipitin reaction.

(4) The control specimens of *Beta vulgaris* (beet), of *Rheum officinale* (rhubarb) and of *Olea europaea* (olive), belonging to three different families, gave always a negative result with each of the immune sera of the other families investigated in this research.

TABLE 1

Precipitin reaction.

PRECIPITINOGEN OF THE	IMMUNE SERUM AGAINST *C. decumana* TITER 1 : 12,800	IMMUNE SERUM AGAINST *P. amygdalus* TITER 1 : 12,800	IMMUNE SERUM AGAINST *Pyrus malus* TITER 1 : 6400	IMMUNE SERUM AGAINST *Lycopersicum esculentum* TITER 1 : 6400	NORMAL RABBIT SERUM
Family: Rutaceae					
Genus: Citrus					
C. decumana	+++	–	–	–	–
C. aurantium	+++	–	–	–	–
C. limonum	+++	–	–	–	–
C. vulgaris	+++	–	–	–	–
C. nobilis	+++	–	–	–	–
Family: Rosaceae					
Subfamily: Prunoideae					
P. amygdalus	–	+++	–	–	–
P. armeniaca	–	+++	–	–	–
P. persica	–	+++	–	–	–
P. domestica	–	+++	–	–	–
Family: Rosaceae					
Subfamily: Pomoideae					
Pyrus malus	–	–	+++	–	–
Pyrus communis	–	–	+++	–	–
Family: Solanaceae					
Lycopersicum esculentum	–	–	–	+++	–
Solanum tuberosum	–	–	–	+++	–
Family: Chenopodiaceae					
Beta vulgaris	–	–	–	–	–
Family: Polygonaceae					
Rheum officinale	–	–	–	–	–
Family: Oleaceae					
Olea europaea	–	–	–	–	–

Note: +++ indicates a positive reaction.
– indicates a negative reaction.

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GAMETOPHYTIC INHERITANCE IN SPHAEROCARPOS. III THE GENETIC UNIFORMITY OF TYPICAL CLONES

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INTRODUCTION

Among the f_1 (gametophytic) progeny of a mating between female and male clones selected as typical for *Sphaerocarpos Donnellii*, a few clones appeared which have hitherto been classed as "possibly atypical." As previously noted (ALLEN 1924, p. 559), such f_1 clones were observed to differ from those classed as "typical" only by displaying at some time or times in their history an unusually large proportion of variant involucres. Similarly doubtful clones of one or of both sexes have appeared among the offspring of matings of tufted ♀ × typical ♂ (ALLEN 1924), typical ♀ × polycladous ♂, and tufted ♀ × polycladous ♂ (ALLEN 1925). The possibility was suggested (ALLEN 1924, p. 561) that the differences between "typical" and "possibly atypical" races might be expressive of new combinations of factors with respect to which the "typical" ancestors differed.

If the phenotypic differences in question have a genetic basis, it would be expected that a mating between one of the doubtful clones and a typical clone of opposite sex would give results different from those of a mating between two typical clones. The experiments here reported were planned to test this possibility.

Improved methods developed within the past three years have, among other advantages, made it possible to secure the germination of a larger proportion of the spores sown. The improvement consists chiefly in maintaining a constant flow of water over the bottoms of enameled pans, each pan fitting closely one of the Wardian cases in which are set the pots of soil containing the spores, spore tetrads, or growing plants. The pots thus stand in a constantly renewed supply of water from one-fourth inch to one inch in depth. In this way the temperatures of the soil as well as of the atmosphere within the case are kept lower than when the pots are set in standing water. The lower temperatures seem to be un-

TABLE 1
Gametophytic offspring of matings 16, 17 and 18.

OFFSPRING (f_1)	TETRADS SOWN SEPARATELY (20 FROM EACH CAPSULE)				TOTALS (TETRADS)
	MATING 16 CAPSULE A	MATING 17		MATING 18 CAPSULE A	
		CAPSULE A	CAPSULE B		
2♀, 2♂	2	3	2	..	7
2♀, 1♂	1	4	1	..	6
2♀	6	3	1	..	10
1♀, 2♂	1	1	3	..	5
1♀, 1♂	1	1	3	1	6
1♀	5	2	3	..	10
2♂	1	3	..	..	4
1♂	2	2	3	..	7
Totals	19	19	16	1	55

Gametophytes possibly atypical: Mating 16, 1♀; mating 17, capsule A, 3♀; capsule B, 2♀. Character undetermined (died young): Mating 17, capsule A, 1♂. All others (61♀'s, 50♂'s) typical.

	TETRADS SOWN BROADCAST				TOTALS (GAMETOPHYTES)	
	MATING 16 CAPSULE B	MATING 17		MATING 18 CAPSULE B		
		CAPSULE B	CAPSULE C			
♀ typical	30	38	63	22	153	161
♀ possibly atypical	3	2	2	..	7	
♀ character undetermined	..	..	1	..	1	
♂ typical	6	36	42	8	92	98
♂ polycladous	..	..	..	1	1	
♂ character undetermined	1	4	..	..	5	
Totals	40	80	108	31		259

All ♀'s (from sowings of both types): typical, 214; possibly atypical, 13; character undetermined, 1; total, 228.

All ♂'s: typical, 142; polycladous, 1; character undetermined, 6; total, 149.

favorable to the development of the algae which have been the most troublesome source of contamination, while they do not noticeably hinder the growth of Sphaerocarpos,—as had been previously shown in experiments devised by Doctor H. W. RICKETT. At first the water used was that obtained by the university pumping station, from Lake Mendota; more recently the culture cases have been connected with the city water supply, which is derived from artesian wells and is practically sterile.

Some of the spore tetrads from each mating were sown separately, and the classification according to sex of the offspring of each tetrad is shown in the first part of table 1. It may be noted here that the results are in harmony with the expectation, based upon previous work, that of each tetrad two spores will develop into female, and two into male gametophytes. Other tetrads were sown broadcast; the classification of their offspring is shown in the second part of table 1.

This work was carried on with the assistance of Mr. J. A. LOUNSBURY, made possible by grants from the research fund of the UNIVERSITY OF WISCONSIN.

DOUBTFUL (POSSIBLY ATYPICAL) ♀ × TYPICAL ♂

Mating 16. 20·337×21·162

Fertilized March 31, 1922. Female plants with mature sporophytes dried June 26. Spore tetrads sown February 2, 1923. Sporelings transplanted to separate pots May 3 to May 19.

Both clones used in this mating were among the offspring of a cross of typical×polycladous (mating 13, ALLEN 1925). Clone 20·337 (the maternal clone in the present mating) showed at times a large proportion of irregularities in involucral form; the paternal clone was at all times classed as typical.

The results of this and of the succeeding matings are shown in table 1. Twenty tetrads from one capsule (A) were sown separately, one to a pot, and the sporelings, after germination, were transplanted separately. The tetrads of a second capsule (B) were scattered upon the soil in a single pot; when the sporelings appeared, they were isolated without reference to their possible tetrad relationships.

Mating 17. 21·153×21·190

Fertilized May 12, 1922. Female plants with mature sporophytes dried July 4. Spore tetrads sown November 3 and November 18. Sporelings isolated March 5 to April 10, 1923.

The maternal clone was an offspring of the same cross (mating 13) of typical×polycladous, that gave rise to both clones used in mating 16. This clone (21·153) was described in a previous paper (ALLEN 1925, p. 8) as one whose frequent aberrancies gave rise to doubt concerning its nature. It was finally classed as "possibly atypical."

The paternal clone (21·190) in the present mating was an offspring of typical×typical (mating 1, ALLEN 1924). Its typical character was at no time doubtful.

Twenty tetrads of capsule A and 20 of capsule B were sown separately. The remaining tetrads of capsule B and all those of capsule C were sown broadcast.

TYPICAL ♀ ×DOUBTFUL ♂

Mating 18. 20·275×20·340

Fertilized March 31, 1922. Female plants with mature sporophytes dried June 23. Tetrads sown February 1, 1923. Sporelings isolated May 15 and May 19.

Both clones used in this mating were offspring of mating 13 (typical ×polycladous). The female clone (20·275) appeared at all times substantially typical. The male clone was the subject of some doubt; at the time of one observation, in particular, it displayed an unusual proportion of aberrant structures, but was finally classed provisionally as typical.

Twenty tetrads from one capsule (A) were sown separately. All the tetrads from a second capsule (B) were sown broadcast.

DISCUSSION

The results of these matings show that "possibly atypical" clones mated with typical clones of opposite sex behave genetically substantially as do typical clones similarly mated. In either case, intraclonal variation among some of the gametophytic offspring is apparently greater than the variation within a single typical clone,—which fact gave rise to the suspicion that some of the apparently more variable clones represent new factorial combinations. This suspicion is not supported by the results here described. The differences noted between "typical" and "possibly atypical" clones seem to be purely phenotypic. The maternal clone (21·153) in mating 17 has displayed perhaps a greater degree of aberrancy than have any of the other doubtful clones of the "possibly atypical" class, yet the proportion among its progeny that can be suspected of being other than typical is no greater than among the progeny of a typical clone similarly mated.

So far as present evidence goes, therefore, the great majority of the plants of *Sphaerocarpos Donnellii* must be considered to be genetically similar,—at least as the species is represented about Sanford and Miami, Florida, the localities from which my material was derived. The only genetically distinct form that occurs, other than very rarely, seems to be the tufted one (ALLEN 1924). Clones of the sort hitherto classed as "possibly atypical" will hereafter, therefore, be treated as typical.

That genetic differences may exist between some of these clones, as well as between other clones ranked as typical, cannot be denied. But such differences, if they exist, are not distinguishable by criteria now available. It is also true that, since strains exist which possess, or at least express, the tufted tendency in a very slight degree, some tufted clones may be classed, from their appearance, even over a considerable period, as typical. Hence, it is to be expected that in an occasional instance the classification of a clone as typical may be found to be erroneous.

The one male clone listed as polycladous among the progeny of mating 18 was apparently the result of a mutation. However, this clone was very small and unhealthy throughout its life, and it is possible, though not probable, that its determination was erroneous. The possibility of an accidental contamination is also not excluded.

LITERATURE CITED

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THE DISTRIBUTION OF THE MAGNITUDES OF THE INTER-MENSUAL CORRELATION COEFFICIENT FOR EGG PRODUCTION IN THE FIRST TWO LAYING YEARS IN THE DOMESTIC FOWL[1]*

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INTRODUCTION

During the past few years the problem of the correlation between the egg record of the several months of the first laying year, the pullet year, of the domestic fowl has received considerable attention.

As far as I am aware, coefficients were first published for the correlation between the October production and that of the other eleven months of the year in connection with investigations on the relationship between body pigmentation and egg record in the White Leghorn breed (HARRIS, BLAKESLEE and WARNER 1917; HARRIS, BLAKESLEE, WARNER and KIRKPATRICK 1917; BLAKESLEE, HARRIS, WARNER and KIRKPATRICK

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[1] The data upon which the present paper is based were collected by Professor H. R. LEWIS, formerly of the NEW JERSEY AGRICULTURAL EXPERIMENT STATION, and his associates. Acknowledgments are due to Professor LEWIS, and to his successors, Professor W. C. THOMPSON and Professor GEO. W. HERVEY, for unfailing generosity and courtesy in facilitating my use of their records. The statistical analysis upon which conclusions are based was largely completed while the writer was a member of the Station for Experimental Evolution of the CARNEGIE INSTITUTION OF WASHINGTON. Funds toward its completion are herewith acknowledged.

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year, or to the intra annual system for the second year. Similarly, we may designate the inter-mensual correlations for the months of the first and of the second year as an inter-annual system for the first and second year.

In the analysis of the three systems of coefficients (which constitute a major system) it becomes necessary to consider the distribution of the magnitudes of the possible correlations between the production of an individual month and those of all other months associated with it as secondary variables. Such a series may be called a minor system of correlations, and may be designated by the month which forms the primary variable in all the combinations.

PRESENTATION AND ANALYSIS OF CONSTANTS

The relationship between the records of the individual months of the year (intra-annual correlation)

It is most convenient to lay the correlations between the records of the individual months of the second year and those between the individual months of the first year side by side in the same table. The constants are arranged symmetrically in table 1. The upper entry of the three which are given for each combination of months is the constant for the second year. The second entry is the constant for the first year. It will be convenient to designate the series of inter-mensual coefficients for one year as a system of correlation coefficients. We have, therefore, to consider the systems for first and second years, respectively.

The inter-mensual, intra-annual correlation coefficients for the first and second year have been abstracted from table 1 and are arranged according to magnitude in table 2. It is clear that the great majority of the constants are low, but generally positive in sign. The average values and the probable errors will be considered below.

As a basis of comparison of the first- and second-year systems, we have determined the differences between the constants for the first and second laying years. These have been taken: constant for second year less con stant for first year. Thus, a positive sign indicates a closer degree of correlation in the second than in the first year.

We have to consider: (a) the possible difference in the average value of the inter-mensual correlation in the two years; (b) the distribution of the individual differences, and the comparison of the differences with their probable errors in order to determine whether certain months have higher correlations in the second than in the first year, or *vice versa*; and (c) the consistency of the magnitudes of homologous inter-mensual corre-

TABLE 1

Correlation coefficients measuring the relationship between the egg production of the individual months of the first year and between the individual months of the second year of the bird's life, and differences between these coefficients.
Upper entry = second year, second entry = first year, lower entry = difference.

	NOVEMBER e_1	DECEMBER e_2	JANUARY e_3	FEBRUARY e_4	MARCH e_5	APRIL e_6	MAY e_7	JUNE e_8	JULY e_9	AUGUST e_{10}	SEPTEMBER e_{11}	OCTOBER e_{12}
November e_1	....	+.4463±.0257 +.6170±.0199 −.1707±.0324	+.0906±.0318 +.3356±.0284 −.2450±.0427	+.1272±.0315 +.2098±.0306 −.0827±.0440	+.0785±.0319 +.2132±.0306 −.1347±.0442	−.0085±.0320 +.1745±.0311 −.1830±.0446	−.0274±.0320 +.1437±.0314 −.1711±.0448	−.0324±.0320 +.0517±.0320 −.0841±.0452	+.0019±.0320 +.0845±.0318 −.0826±.0452	+.1161±.0316 +.1044±.0317 +.0116±.0448	+.2524±.0300 +.1586±.0312 +.0938±.0433	+.3396±.0284 +.1290±.0315 +.2106±.0424
December e_2	+.4463±.0257 +.6170±.0199 −.1707±.0324	....	+.2299±.0304 +.6309±.0193 −.4010±.0360	+.2040±.0307 +.2597±.0299 −.0557±.0429	+.1167±.0316 +.1292±.0315 −.0125±.0446	+.0235±.0320 +.1086±.0317 −.0851±.0450	−.0373±.0320 +.1188±.0316 −.1561±.0450	−.0212±.0320 +.0675±.0319 −.0888±.0452	+.0223±.0320 +.1089±.0317 −.0866±.0450	+.0988±.0317 +.2030±.0307 −.1042±.0442	+.1635±.0312 +.2792±.0296 −.1157±.0430	+.1772±.0310 +.2480±.0301 −.0708±.0432
January e_3	+.0906±.0318 +.3356±.0284 −.2450±.0427	+.2299±.0304 +.6309±.0193 −.4010±.0360	....	+.3885±.0272 +.4201±.0264 −.0316±.0379	+.2978±.0292 +.2238±.0304 +.0741±.0422	+.0343±.0320 +.1622±.0312 −.1279±.0447	+.0281±.0320 +.1304±.0315 −.1023±.0449	+.0566±.0319 +.0398±.0320 +.0168±.0452	+.1095±.0317 +.0929±.0318 +.0167±.0449	+.1847±.0310 +.1604±.0312 +.0243±.0440	+.2736±.0297 +.3046±.0291 −.0310±.0415	+.1715±.0311 +.2852±.0294 −.1137±.0428
February e_4	+.1272±.0315 +.2098±.0306 −.0827±.0440	+.2040±.0307 +.2597±.0299 −.0557±.0429	+.3885±.0272 +.4201±.0264 −.0316±.0379		+.4587±.0253 +.3973±.0270 +.0615±.0370	+.0947±.0318 +.2413±.0302 −.1466±.0438	+.0012±.0320 +.1329±.0315 −.1317±.0449	+.1527±.0313 +.0325±.0320 +.1203±.0448	+.1326±.0315 +.1455±.0314 −.0129±.0444	+.2033±.0307 +.1773±.0310 +.0260±.0437	+.2602±.0299 +.1957±.0308 +.0644±.0429	+.1557±.0313 +.2066±.0307 −.0510±.0438
March e_5	+.0785±.0319 +.2132±.0306 −.1347±.0442	+.1167±.0316 +.1292±.0315 −.0125±.0446	+.2978±.0292 +.2238±.0304 +.0741±.0422	+.4587±.0253 +.3973±.0270 +.0615±.0370	....	+.4094±.0267 +.6253±.0195 −.2158±.0331	+.2965±.0292 +.3804±.0274 −.0839±.0401	+.2673±.0298 +.2472±.0301 +.0201±.0423	+.2735±.0297 +.2206±.0305 +.0529±.0425	+.2514±.0300 +.1886±.0309 +.0628±.0431	+.2445±.0301 +.1154±.0316 +.1291±.0437	+.1590±.0312 +.1297±.0315 +.0293±.0444
April e_6	−.0085±.0320 +.1745±.0311 −.1830±.0446	+.0235±.0320 +.1086±.0317 −.0851±.0450	+.0343±.0320 +.1622±.0312 −.1279±.0447	+.0947±.0318 +.2413±.0302 −.1466±.0438	+.4094±.0267 +.6253±.0195 −.2158±.0331	. ...	+.5920±.0208 +.5765±.0214 +.0154±.0299	+.4332±.0260 +.2762±.0296 +.1570±.0394	+.3224±.0287 +.2136±.0306 +.1089±.0420	+.2032±.0307 +.1904±.0309 +.0128±.0436	+.1319±.0315 +.1509±.0313 −.0190±.0444	+.0826±.0318 +.1144±.0316 −.0318±.0449
May e_7	−.0274±.0320 +.1437±.0314 −.1711±.0448	−.0373±.0320 +.1188±.0316 −.1561±.0450	+.0281±.0320 +.1304±.0315 −.1023±.0449	+.0012±.0320 +.1329±.0315 −.1317±.0449	+.2965±.0292 +.3804±.0274 −.0839±.0401	+.5920±.0208 +.5765±.0214 +.0154±.0299	....	+.6463±.0187 +.5384±.0228 +.1079±.0294	+.4443±.0257 +.3208±.0288 +.1234±.0386	+.2757±.0296 +.2430±.0302 +.0327±.0423	+.1797±.0310 +.1379±.0314 +.0418±.0442	+.0723±.0319 +.1070±.0317 −.0347±.0449
June e_8	−.0324±.0320 +.0517±.0320 −.0841±.0452	−.0212±.0320 +.0675±.0319 −.0888±.0452	+.0566±.0319 +.0398±.0320 +.0168±.0452	+.1527±.0313 +.0325±.0320 +.1203±.0448	+.2673±.0298 +.2472±.0301 +.0201±.0423	+.4332±.0260 +.2762±.0296 +.1570±.0394	+.6463±.0187 +.5384±.0228 +.1079±.0294	....	+.5926±.0208 +.5169±.0235 +.0758±.0314	+.3671±.0277 +.3610±.0279 +.0060±.0393	+.2202±.0305 +.1731±.0311 +.0471±.0435	+.0786±.0319 +.0463±.0320 +.0323±.0451
July e_9	+.0019±.0320 +.0845±.0318 −.0826±.0452	+.0223±.0320 +.1089±.0317 −.0866±.0450	+.1095±.0317 +.0929±.0318 +.0167±.0449	+.1326±.0315 +.1455±.0314 −.0129±.0444	+.2735±.0297 +.2206±.0305 +.0529±.0425	+.3224±.0287 +.2136±.0306 +.1089±.0420	+.4443±.0257 +.3208±.0288 +.1234±.0386	+.5926±.0208 +.5169±.0235 +.0758±.0314	.. . .	+.5380±.0228 +.5764±.0214 −.0383±.0313	+.3474±.0282 +.1757±.0311 +.1717±.0419	+.1841±.0310 +.0348±.0320 +.1492±.0445
August e_{10}	+.1161±.0316 +.1044±.0317 +.0116±.0448	+.0988±.0317 +.2030±.0307 −.1042±.0442	+.1847±.0310 +.1604±.0312 +.0243±.0440	+.2033±.0307 +.1773±.0310 +.0260±.0437	+.2514±.0300 +.1886±.0309 +.0628±.0431	+.2032±.0307 +.1904±.0309 +.0128±.0436	+.2757±.0296 +.2430±.0302 +.0327±.0423	+.3671±.0277 +.3610±.0279 +.0060±.0393	+.5380±.0228 +.5764±.0214 −.0383±.0313	.. ..	+.6411±.0189 +.4548±.0254 +.1862±.0317	+.2729±.0297 +.2260±.0304 +.0469±.0425
September e_{11}	+.2524±.0300 +.1586±.0312 +.0938±.0433	+.1635±.0312 +.2792±.0296 −.1157±.0430	+.2736±.0297 +.3046±.0291 −.0310±.0415	+.2602±.0299 +.1957±.0308 +.0644±.0429	+.2445±.0301 +.1154±.0316 +.1291±.0437	+.1319±.0315 +.1509±.0313 −.0190±.0444	+.1797±.0310 +.1379±.0314 +.0418±.0442	+.2202±.0305 +.1731±.0311 +.0471±.0435	[illegible].3474±.0282 [illegible].1757±.0311 [illegible]1717±.0419	+.6411±.0189 +.4548±.0254 +.1862±.0317	... .	+.6178±.0198 +.7065±.0161 −.0887±.0255
October e_{12}	+.3396±.0284 +.1290±.0315 +.2106±.0424	+.1772±.0310 +.2480±.0301 −.0708±.0432	+.1715±.0311 +.2852±.0294 −.1137±.0428	+.1557±.0313 +.2066±.0307 −.0510±.0438	+.1590±.0312 +.1297±.0315 +.0293±.0444	+.0826±.0318 +.1144±.0316 −.0318±.0449	+.0723±.0319 +.1070±.0317 −.0347±.0449	+.0786±.0319 +.0463±.0320 +.0323±.0451	+.[illegible]841±.0310 +.0[illegible]48±.0320 +.1[illegible]2±.0445	+.2729±.0297 +.2260±.0304 +.0469±.0425	+.6178±.0198 +.7065±.0161 −.0887±.0255	....

TABLE 2

Distribution of magnitudes of inter-mensual correlation coefficients for first and for second laying year.

RANGE OF CORRELATION	FREQUENCY FIRST YEAR	FREQUENCY SECOND YEAR
− .001 to − .050		5
+ .001 to + .050	4	6
+ .051 to + .100	4	8
+ .101 to + .150	15	6
+ .151 to + .200	11	9
+ .201 to + .250	12	6
+ .251 to + .300	4	10
+ .301 to + .350	3	3
+ .351 to + .400	3	2
+ .401 to + .450	1	4
+ .451 to + .500	1	1
+ .501 to + .550	2	1
+ .551 to + .600	2	2
+ .601 to + .650	3	3
+ .651 to + .700		
+ .701 to + .750	1	
Total	66	66

lations in the systems for the first and second years, respectively,—most conveniently measured in terms of the correlation between the constants of the system of coefficients for the first year and second year.

Comparing first the mean values of the inter-mensual correlation in the two years, we have

For second year,	0.2199 ± .0146
For first year,	0.2390 ± .0135
Difference,	+0.0191 ± .0093

The probable error of the difference between the mean correlation for the months of the first and for the months of the second year may be determined by computing the standard deviation of the differences themselves as shown in table 1, or from the frequency distribution of the differences grouped in classes of uniform interval as given in table 3, or from the formula involving the correlation between the two systems of intra-annual correlation coefficients,

$$\sigma^2_{(r_{iieiie}-r_{ieie})}=\sigma^2_{r_{iieiie}}+\sigma^2_{r_{ieie}}-2r_{r_{iieiie}r_{ieie}}\sigma_{r_{iieiie}}\sigma_{r_{ieie}}$$

where $r_{r_{iieiie}r_{ieie}}$ is the coefficient measuring the correlation between the homologous values of the two systems of intra-annual inter-mensual correlation coefficients, as indicated by the subscripts *i* and *ii* for the monthly egg productions, *e*, of the first and second years. The coefficient required is given below.

Numerically, the average of the correlations for the second year is slightly lower than that of those for the first year, but the difference is less than twice as large as its probable error and cannot be considered definitely significant.

The frequency distributions of the differences, and the ratios of these differences to their individual probable errors are set forth in tables 2 and 3. Table 2 gives the differences in the actual magnitudes of the correlation coefficients. We note that the differences are for the most part relatively small. Forty-one of the differences fall between −.100 and +.100. Twenty-five of the coefficients fall outside this range. Of these, ten are positive, while fifteen are negative.

TABLE 3

Difference in the correlation coefficients for the two years.

DIFFERENCE IN CORRELATION (SECOND YEAR—FIRST YEAR)	FREQUENCY
−.401 to −.450	1
−.351 to −.400	.
−.301 to −.350	..
−.251 to −.300	
−.201 to −.250	2
−.151 to −.200	4
−.101 to −.150	8
−.051 to −.100	11
−.001 to −.050	8
+.001 to +.050	15
+.051 to +.100	7
+.101 to +.150	6
+.151 to +.200	3
+.201 to +.250	1
Total	66

TABLE 4

Ratios of differences in correlation to probable errors.

RATIO TO PROBABLE ERROR AND SIGN OF DIFFERENCE IN CORRELATION	FREQUENCY
−11.001 to −12.000	1
−10.001 to −11.000	..
− 9.001 to −10.000	.
− 8.001 to − 9.000	
− 7.001 to − 8.000	..
− 6.001 to − 7.000	1
− 5.001 to − 6.000	2
− 4.001 to − 5.000	1
− 3.001 to − 4.000	5
− 2.001 to − 3.000	7
− 1 001 to − 2.000	10
− 0.001 to − 1.000	7
+ 0.001 to + 1.000	13
+ 1.001 to + 2.000	7
+ 2.001 to + 3.000	5
+ 3.001 to + 4.000	4
+ 4 001 to + 5.000	2
+ 5.001 to + 6.000	1
Total	66

Turning to table 4 which shows the ratios of the differences in the coefficients for the two years, to their probable errors, we note that only

20 of the ratios fall between −1.0 and +1.0, whereas 33 should fall between these limits if variations in the differences were due to the probable errors of random sampling. Thus, it appears that certain of the differences are statistically significant with regard to their probable errors. We must, therefore, conclude that while on the average the constants of inter-mensual correlations for the first and second year are of about the same average magnitude, there are combinations of months in which the two constants differ significantly.

An interesting case is that of the conspicuous difference noted at the top of table 3. This is the difference in the coefficient for December and January. The coefficient has the value +.230 in the second year, whereas in the first year it has the value +.631. The difference is −.401 ± .036. This must be regarded as clearly significant in comparison with its probable error. If we examine other coefficients in this portion of the year we find that similar, although not so marked, differences are found in the correlations between November and December, and November and January.

A probable explanation lies in the fact that different numbers of birds are laying in these months in the two years. Turning back to the tables of an earlier paper (HARRIS and LEWIS 1922, tables 13 to 15) we may calculate the percentage of these birds which did lay in each of the first three months of the contest in first and second years, respectively. The results show that in November of the second year 78.6 percent of the birds did not lay, whereas in November of the first year only 19.4 percent did not lay. In December of the second year, 74.9 percent of the birds did not lay, whereas in December of the first year only 22.3 percent of the birds did not lay. Finally, in January of the second year, 27.1 percent of the birds did not lay, whereas in January of the first year only 20.5 percent of the birds did not lay.

We may now consider the same kind of evidence, but expressed in a different way. The time of beginning of laying in the two years have been tabulated elsewhere (HARRIS and LEWIS 1923, diagrams 1 and 2). We note that in the first year the great bulk of the birds began to lay very early in November. In the second year this was not the case.

It is altogether probable that the smaller proportion of birds which were really expressing their innate capacities in the early months of the second year, results in the low values of the correlations between the earlier months of this year as compared with those found in the first year. It has already been shown (HARRIS, BLAKESLEE and KIRKPATRICK 1918, pp. 49 to 56) that groups of birds which appear identical on the basis of an alternative system of classification are in reality heterogeneous, and that supplementary criteria may be used in dividing these groups into finer categories.

While the explanation suggested is probably valid for the particular differences for which it is offered, it cannot be applied to some of the other differences which are apparently significant in comparison with their probable errors. Further discussion of individual discrepancies between the results for the first and second years should probably await the analysis and comparative examination of larger series of data.

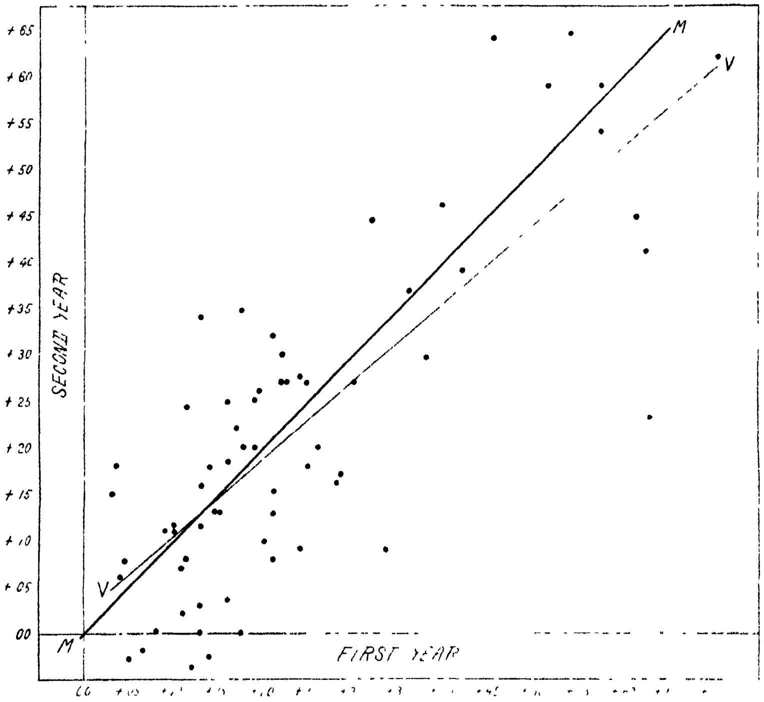

Diagram 1.—Scatter diagram showing the relationship between the values of homologous inter-mensual intra-annual correlations for the first and second years.

The problem of the general consistency of the homologous coefficients of the two years may be most readily solved, on the basis of the data available, up to the present stage of our discussion, and the results of the solution most clearly expressed by the determination of a coefficient of correlation measuring the relationship between the two systems of coefficients. The problem will be taken up from another side later (see page 104).

Working from the entries of table 1 we find a coefficient of correlation between the homologous constants for the first and second year of

$$r_{r_{\mathrm{I}e\mathrm{I}e'}\, r_{\mathrm{II}e\mathrm{II}e'}} = .7839 \pm .0320$$

Thus, there is a very high, but by no means perfect, correlation between the systems of constants for the two years.

The closeness of this interrelationship may be shown graphically by representing the positions of the individual pairs of correlations on a plane. On diagram 1 the scale of abscissae represents the magnitudes of the inter-mensual correlations for the first year, while the scale of ordinates represents the magnitudes of the inter-mensual correlations for the second year.

On this diagram the heavy line, M—M, represents equality of correlation for the systems of the first and second year. This line divides the solid dots, which represent the correlation coefficients, into approximately equal groups, thus showing that the difference between the coefficients for the first and the second year is not large.

If the average value and the variability of the values of the coefficients for the two years were the same, a line fitted to the swarm of constants should coincide with the line M—M. The equation to the line is

$$r_{\mathrm{II}e\mathrm{II}e'} = 0.0177 + 0.8459\, r_{\mathrm{I}e\mathrm{I}e'}$$

The equation represented by the line V—V is a close approximation to the line M—M.

The relationship between the records of the individual months of the first and second year (inter-annual correlation)

The constants of this system of coefficients have already been published (HARRIS and LEWIS 1922 a, table 4). In subsequent sections they will be considered in their relationship to the intra-annual coefficients and to the distribution of the magnitudes of the coefficients for the whole two-year period.

The relative magnitudes of intra-annual and inter-annual correlation coefficients

We have first to consider the problem of the relative magnitude of the intra-annual and the inter-annual correlation coefficients.

To make the matter quite clear by the use of an illustration, our problem is to determine the relative magnitude of the correlation between the production of November of the first year and December of the second year (the inter-annual correlation) as compared with that between November and December of the first year (the intra-annual correlation)

TABLE 5, PART 1

Comparison between intra-annual inter-mensual correlation coefficients and inter-annual inter-mensual correlation coefficients measuring the relationship between the egg records of the months of the first and second laying years. Under each correlation is given the value of the coefficient divided by its probable error.

Month of first year for inter-annual correlations; month of first or second year for intra-annual correlations	NOVEMBER e_1	DECEMBER e_2	JANUARY e_3	FEBRUARY e_4	MARCH e_5	APRIL e_6
November e_1	.. .	−.3717 ± .0409 9.08 −.5423 ± .0375 14.4	+.0721 ± .0445 1.62 −.1729 ± .0422 4.09	+.0782 ± .0440 1.78 −.0045 ± .0434 0.10	+.0901 ± .0445 2.02 −.0446 ± .0437 1.02	+.0495 ± .0453 1.09 −.1336 ± .0446 2.99
December e_2	−.2446 ± .0401 6.11 −.4153 ± .0366 11.3	....	−.0104 ± .0430 0.24 −.4114 ± .0361 11.4	+.0675 ± .0427 1.58 +.0118 ± .0421 0.28	+.0338 ± .0445 0.76 +.0212 ± .0444 0.48	−.0051 ± .0453 0.11 −.0902 ± .0453 2.00
January e_3	+.1124 ± .0442 2.54 −.1325 ± .0419 3.17	−.0586 ± .0435 1.35 −.4596 ± .0366 12.6	....	−.0910 ± .0399 2.28 −.1226 ± .0394 3.11	−.1323 ± .0428 3.10 −.0582 ± .0436 1.34	−.0766 ± .0453 1.69 −.2045 ± .0447 4.58
February e_4	+.0334 ± .0444 0.75 −.0493 ± .0437 1.13 0	−.0036 ± .0435 0.08 −.0593 ± .0429 1.38	−.0569 ± .0394 1.44 −.0885 ± .0389 2.28	.. .	−.2215 ± .0394 5.62 −.1600 ± .0405 3.95	−.1296 ± .0451 2.87 −.2761 ± .0440 6.28
March e_5	−.0041 ± .0451 0 09 −.1388 ± .0442 3.14	−.0951 ± .0450 2.11 −.1077 ± .0449 2.40	−.1469 ± .0428 3.43 −.0729 ± .0437 1.67	−.2206 ± .0394 5.59 −.1591 ± .0405 3.93	....	−.3156 ± .0415 7.61 −.5315 ± .0373 14.3
April e_6	+.0410 ± .0453 0.91 −.1420 ± .0446 3.18	−.0525 ± .0453 1.16 −.1376 ± .0450 3.05	+.0133 ± .0452 0.29 −.1146 ± .0447 2.57	+.0083 ± .0449 0.19 −.1382 ± .0438 3.16	−.0919 ± .0393 2.34 −.3077 ± .0348 8.84	....

Month of second year for inter-annual correlations; month of first or second year for intra-annual correlations.

TABLE 5, PART 2

Month of first year for inter-annual correlations; month of first or second year for intra-annual correlations

	MAY e_7	JUNE e_8	JULY e_9	AUGUST e_{10}	SEPTEMBER e_{11}	OCTOBER e_{12}
November e_1	+.1038 ± .0452 2.30	+ 0856 ± .0452 1.89	+ 0201 ± 0453 0.44	−.0882 ± .0450 1.96	−.1269 ± .0435 2 91	−.2464 ± .0426 5.79
	−.0673 ± .0447 1.50	+.0015 ± 0452 0.03	−.0625 ± .0452 1.38	−.0766 ± .0451 1.70	−.0331 ± .0444 0.75	−.0358 ± .0448 0.80
December e_2	+.0748 ± 0453 1.65	+.0907 ± .0452 2 01	+.0665 ± .0451 1.47	+.0493 ± .0446 1.10	+.0977 ± .0432 2 26	+.0130 ± .0438 0.30
	−.0813 ± .0450 1.81	+.0020 ± .0451 0.04	− 0200 ± 0449 0.45	−.0549 ± .0439 1.25	−.0180 ± .0420 0.43	−.0578 ± .0431 1.34
January e_3	−.0665 ± .0453 1.47	−.0282 ± 0452 0.62	+.0149 ± 0447 0.33	+ 0161 ± .0436 0.37	+.0472 ± .0423 1.14	+.0522 ± .0435 1.20
	−.1688 ± .0449 3.76	− 0115 ± .0453 0.25	+.0316 ± 0448 0 71	+.0404 ± .0438 0.92	+.0163 ± .0409 0.40	−.0615 ± .0424 1.45
February e_4	−.0664 ± .0452 1.47	−.1279 ± .0448 2.86	−.0337 ± .0447 0 75	−.0831 ± .0441 1.89	−.0478 ± .0428 1.12	−.0543 ± .0445 1.22
	−.1981 ± .0448 4.42	− 0076 ± .0453 0.17	−.0466 ± .0446 1.04	−.0571 ± .0443 1.29	+ 0166 ± .0434 0.38	−.1052 ± .0441 2.38
March e_5	−.2176 ± .0432 5.03	−.1777 ± 0435 4 08	−.1761 ± .0434 4.05	−.1113 ± .0434 2.56	−.0834 ± .0434 1.92	−.0775 ± .0446 1.74
	−.3015 ± .0420 7.17	−.1577 ± .0438 3 60	−.1232 ± 0440 2 80	−.0485 ± .0441 1.10	+ 0457 ± .0444 1.03	− 0482 ± .0448 1.08
April e_6	−.4146 ± .0374 11.1	−.2772 ± .0407 6.81	−.2163 ± 0428 5.06	− 0923 ± .0441 2 09	−.0156 ± 0446 0.35	−.0197 ± 0451 0.44
	−.3991 ± .0377 10.6	−.1202 ± .0431 2.79	−.1074 ± .0440 2.44	− 0795 ± .0442 1.80	−.0345 ± .0445 0.78	−.0514 ± .0449 1.14

Month of second year for inter-annual correlations; month of first or second year for intra-annual correlations

TABLE 5, PART 3

Comparison between intra-annual inter-mensual correlation coefficients and inter-annual inter-mensual correlation coefficients measuring the relationship between the egg records of the months of the first and second laying years. Under each correlation is given the value of the coefficient divided by its probable error.

Month of first year for inter-annual correlations; month of first or second year for intra-annual correlations	NOVEMBER e_1	DECEMBER e_2	JANUARY e_3	FEBRUARY e_4	MARCH e_5	APRIL e_6
May	+.0440 ± .0453 0.97	+.0108 ± .0453 0.24	+.0058 ± .0453 0.13	+.0268 ± .0453 0.59	−.1043 ± .0425 2.45	−.3110 ± .0361 8.61
e_7	−.1271 ± .0449 2.83	−.1453 ± .0450 3.23	−.0965 ± .0449 2.15	−.1049 ± .0449 2.34	−.1882 ± .0413 4.56	−.2956 ± .0365 8.11
June	+.0147 ± .0453 0.32	+.0378 ± .0453 0.83	+.0383 ± .0450 0.85	−.1345 ± .0448 3.00	−.0830 ± .0429 1.93	−.1646 ± .0395 4.16
e_8	−.0694 ± .0453 1.53	−.0510 ± .0452 1.13	+.0551 ± .0451 1.22	−.0143 ± .0453 0.32	−.0630 ± .0432 1.46	−.0075 ± .0420 0.18
July	−.0462 ± .0453 1.02	+.0130 ± .0453 0.29	+.0866 ± .0442 1.96	−.0685 ± .0448 1.53	−.0587 ± .0426 1.38	−.1148 ± .0420 2.73
e_9	−.1288 ± .0451 2.86	−.0736 ± .0450 1.64	+.1033 ± .0443 2.33	−.0814 ± .0448 1.82	−.0058 ± .0432 0.14	−.0059 ± .0433 0.14
August	−.0347 ± .0449 0.77	−.0096 ± .0449 0.21	+.0161 ± .0436 0.37	−.0851 ± .0441 1.93	−.0853 ± .0433 1.97	−.0625 ± .0439 1.42
e_{10}	−.0230 ± .0449 0.51	−.1138 ± .0442 2.57	+.0404 ± .0438 0.92	−.0591 ± .0443 1.33	−.0225 ± .0439 0.51	−.0497 ± .0441 1.13
September	−.1846 ± .0438 4.21	−.2074 ± .0447 4.64	−.1471 ± .0433 3.40	−.1288 ± .0434 2.97	−.1376 ± .0437 3.15	−.1017 ± .0449 2.26
e_{11}	−.0908 ± .0447 2.03	−.3231 ± .0435 7.42	−.1781 ± .0429 4.15	−.0644 ± .0441 1.46	−.0085 ± .0448 0.19	−.1206 ± .0448 2.69
October	+.0498 ± .0393 1.27	−.1580 ± .0446 3.54	−.0462 ± .0443 1.04	+.0456 ± .0439 1.04	−.0650 ± .0446 1.46	−.0723 ± .0452 1.60
e_{12}	+.2604 ± .0416 6.26	−.2288 ± .0439 5.21	−.1599 ± .0432 3.70	−.0054 ± .0434 0.12	−.0357 ± .0447 0.80	−.1041 ± .0450 2.31

Month of second year for inter-annual correlations; month of first or second year for intra-annual correlations

TABLE 5, PART 4

Month of first year for inter-annual correlations; month of first or second year for intra-annual correlations	NOVEMBER e_7	DECEMBER e_8	JANUARY e_9	FEBRUARY e_{10}	MARCH e_{11}	APRIL e_{12}
May e_7	. . . .	− .3272 ± .0343 9.54 − .2193 ± .0367 5.98	− .2126 ± .0398 5.35 − .0891 ± .0418 2.13	− .0777 ± .0427 1 82 − .0450 ± 0431 1.04	− 0372 ± .0444 0 84 + 0046 ± .0444 0 10	+ .0073 ± .0451 0.16 − .0274 ± .0449 0.61
June e_8	− .2534 ± .0329 7.70 − .1456 ± .0354 4.11	.	− 2870 ± 0357 8 03 − 2113 ± 0374 5 65	− .0860 ± .0405 2 12 − .0800 ± 0406 1 97	− 0182 ± .0433 0 42 + .0288 ± .0437 0 66	− .0237 ± .0451 0 53 + .0086 ± .0452 0.19
July e_9	− .0801 ± 0379 2.11 + 0433 ± 0400 1.08	− 2057 ± 0343 6 00 − 1299 ± .0360 3.61	. . . .	− .2826 ± 0376 7 51 − 3210 ± .0368 8.72	− .1291 ± 0415 3 11 + .0426 ± 0436 0.98	− .1168 ± .0444 2.63 + 0325 ± .0452 0.72
August e_{10}	− .0087 ± .0420 0 21 + .0240 ± 0424 0.57	− .0899 ± .0406 2 22 − .0838 ± .0406 2.06	− .1728 ± 0359 4.81 − .2112 ± .0351 6 02	. . . . . .	− .2756 ± .0336 8.21 − 0784 ± .0376 2.37	− .0182 ± .0422 0.43 + .0286 ± .0427 0.67
September e_{11}	− .1637 ± .0446 3 67 − 1219 ± 0449 2.72	− .1720 ± .0442 3.89 − 1249 ∓ .0446 2 80	− .0916 ± .0411 2 23 +0800 ± .0432 1.85	− .3056 ± .0341 8 95 − .1194 ± .0381 3.13	. . .	− .2273 ± .0336 6.76 − .3160 ± .0315 10.0
October e_{12}	− .1055. ± 0452 2 34 − 1402 ± 0450 3 11	− .0730 ± .0452 1.62 − .0407 ± 0453 0 90	− .0555 ± 0442 1 26 + .0938 ± .0449 2 09	− 0078 ± .0420 0 19 + 0391 ± 0426 0 92	.1379. ± 0316 4 36 .2266 ± 0294 7.70	. . . .

Month of second year for inter-annual correlations; month of first or second year for intra-annual correlations

on the one hand, and November and December of the second year (the intra-annual correlation) on the other. Similar comparisons are to be made for each of 132 of the 144 possible (inter-annual) correlations between the productions of the individual months of the first and second year, and of the homologous combinations within the first and within the second year.

The differences and the probable errors of the differences between the inter-annual and the intra-annual coefficients are given with their probable errors and their ratios to their probable errors in table 5.[2] In this table the upper entry shows the differences between the inter-annual correlations between the individual monthly productions of the first year and those of the second year and the intra-annual correlations of the second year. The lower entry shows the differences between the inter-annual correlations and the intra-annual correlations for the first year. The differences are so taken that a positive sign indicates that the correlation between the productions of the first and second year is higher than that between the individual months of the first or of the second year, as the case may be. A negative sign indicates that the correlation between the months of the different years is lower than that between the months of the same, either first or second.

We may first draw the comparisons on the basis of the mean values for the three series. The constants are:

Inter-annual correlation, first and second year	$+.1439 \pm .0332$
Intra-annual correlation, first year	$+.2390 \pm .0135$
Difference	$-.0951 \pm .0072$
Intra-annual correlation, second year	$+.2199 \pm .0146$
Difference	$-.0760 \pm .0065$

[2] For 12 of the 144 coefficients measuring the relationship between the productions of the months of the first and second year there are no comparable coefficients for the relationship within the same year, since there are only $12 \times 11 = 132$ possible combinations of the months of each year, instead of the $12 \times 12 = 144$ combinations for the two different years.

Table 5 has the same general form as table 1, but because of its large size it has been broken up into four sections as follows:

Part 1
First year: November–April
Second year: November–April

Part 2
First year: November–April
Second year: May–October

Part 3
First year: May–October
Second year: November–April

Part 4
First year: May–October
Second year: May–October

The average and standard deviation of the inter-annual correlation coefficient has been calculated on the basis of the 132 coefficients for which comparison with the intra-annual correlations may be made. The probable errors of the differences between inter-annual and intra-annual coefficients have been determined from the standard deviations of the differences as shown in the frequency distributions of table 6.

TABLE 6

Differences between inter-annual and intra-annual correlation coefficients
Differences taken: inter-annual—intra-annual.

DIFFERENCE IN CORRELATION	FREQUENCY FIRST YEAR	FREQUENCY SECOND YEAR
−.501 to −.550	2	.
−.451 to −.500	1	..
−.401 to −.450	2	1
−.351 to −.400	1	1
−.301 to −.350	5	4
−.251 to −.300	2	5
−.201 to −.250	6	10
−.151 to −.200	9	8
−.101 to −.150	26	17
−.051 to −.100	28	30
−.001 to −.050	26	19
+.001 to +.050	19	24
+.051 to +.100	3	11
+.101 to +.150	1	2
+.151 to +.200	..	..
+.201 to +.250		..
+.251 to +.300	1	..
Totals	132	132

The differences given above have been calculated from ungrouped values of the coefficients, not from the grouped values as shown in table 6. The values calculated from the grouped constants of the table are, of course, sensibly the same.

The differences are both negative in sign, thus indicating that the inter-annual correlations are somewhat lower than the homologous intra-annual coefficients. The difference between inter-annual and first-year intra-annual is 13.2 times as large as its probable error, and the difference between inter-annual and second-year intra-annual is 11.7 times as large as its probable error. Thus, both differences may be reasonably considered statistically significant.

As a second means of comparison the frequency distribution of the differences may be considered. These are shown in table 6. The frequency distribution of the ratios of these differences to their probable errors are shown in table 7.

The distribution of the differences in correlation in table 6 shows that by far the greater number of the differences are negative in sign for both of the years.

If we consider that differences ranging from −.050 to +.050 may be regarded essentially zero,[3] we note that for the first-year comparison there are 82 negative differences as compared with 5 which are positive, while for the second year there are 76 negative differences as compared with 13 which are positive in sign.

TABLE 7

Ratios of differences between inter-annual and intra-annual correlation coefficients to their probable errors.

RATIO TO DIFFERENCE AND SIGN OF DIFFERENCE	FREQUENCY FIRST YEAR	FREQUENCY SECOND YEAR
−14.001 to −15.000	2	
−13.001 to −14.000		
−12.001 to −13.000	1	
−11.001 to −12.000	2	1
−10.001 to −11.000	2	
− 9.001 to −10.000		2
− 8.001 to − 9.000	3	4
− 7.001 to − 8.000	3	3
− 6.001 to − 7.000	2	4
− 5.001 to − 6.000	3	6
− 4.001 to − 5.000	6	7
− 3.001 to − 4.000	15	9
− 2.001 to − 3.000	22	18
− 1.001 to − 2.000	28	25
− 0.001 to − 1.000	19	16
+ 0.001 to + 1.000	17	19
+ 1.001 to + 2.000	4	13
+ 2.001 to + 3.000	2	5
+ 3.001 to + 4.000		
+ 4.001 to + 5.000		
+ 5.001 to + 6.000		
+ 6.001 to + 7.000	1	
Totals	132	132

---

[3] The probable errors of the differences are of the order 0.040 to 0.045 for the first year and 0.039 to 0.045 for the second year. Thus, the range −.050 to +.050 is approximately coextensive with the probable error for coefficients of this magnitude.

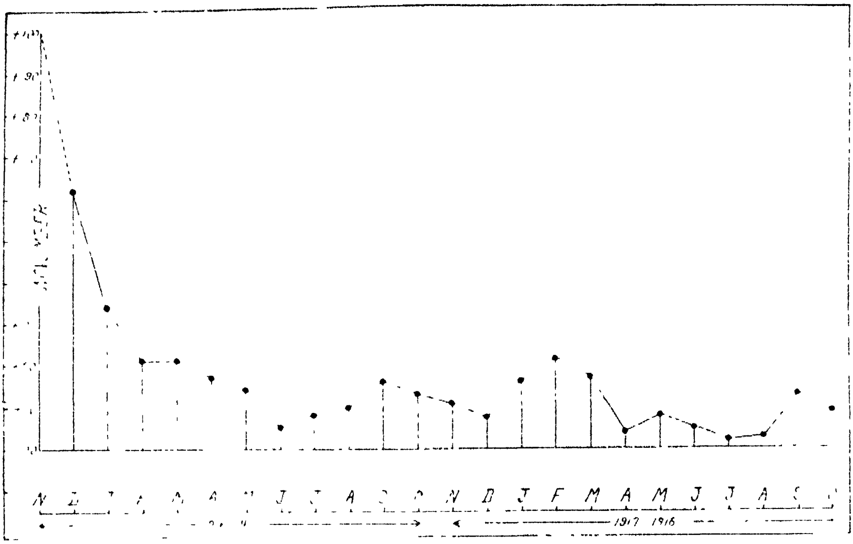

DIAGRAM 2.—Distribution of the magnitudes of the inter-mensual correlation coefficients for November of the first year.

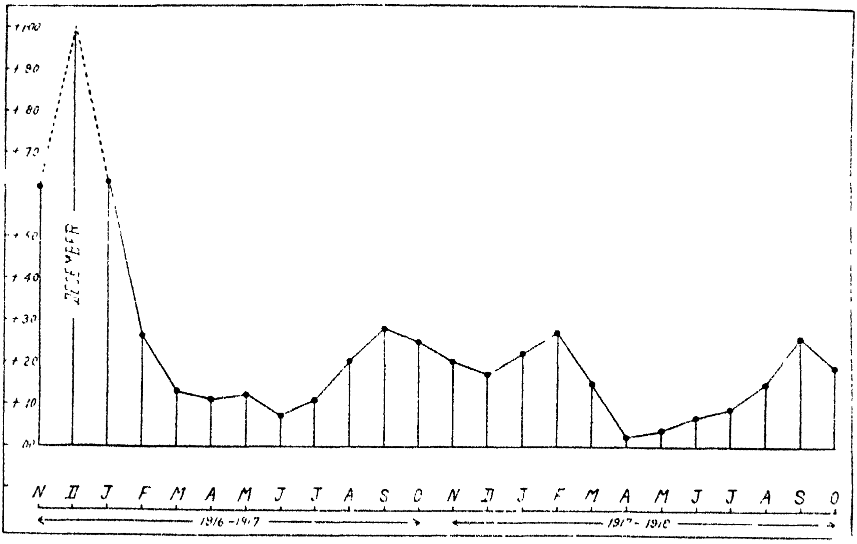

DIAGRAM 3.—Distribution of the magnitudes of the inter-mensual correlation coefficients for December of the first year.

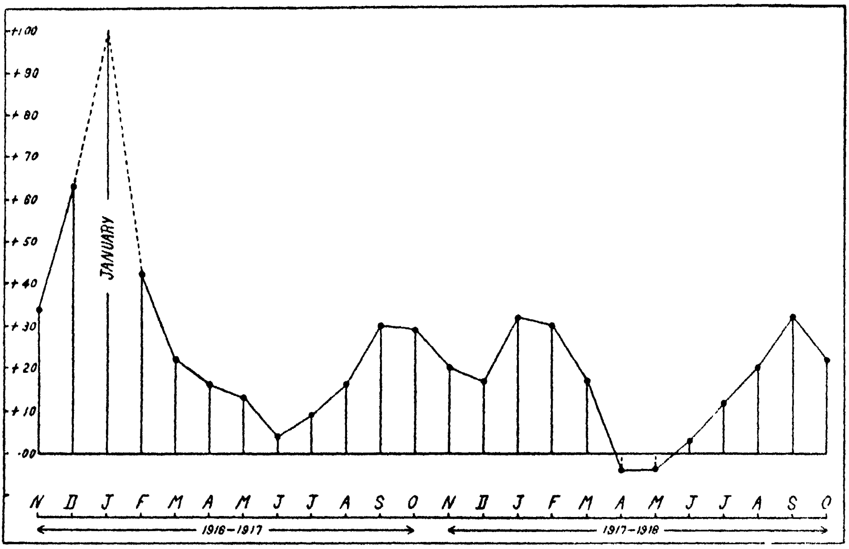

DIAGRAM 4.—Distribution of the magnitudes of the inter-mensual correlation coefficients for January of the first year.

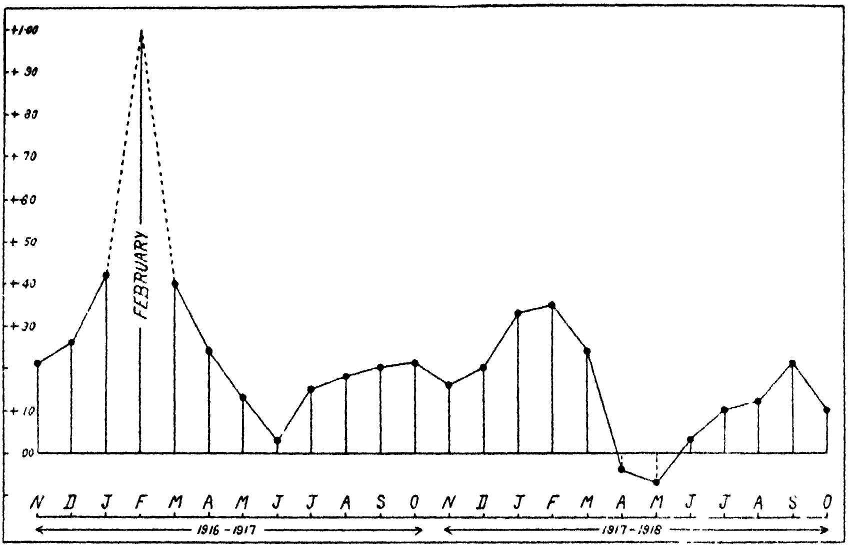

DIAGRAM 5.—Distribution of the magnitudes of the inter-mensual correlation coefficients for February of the first year.

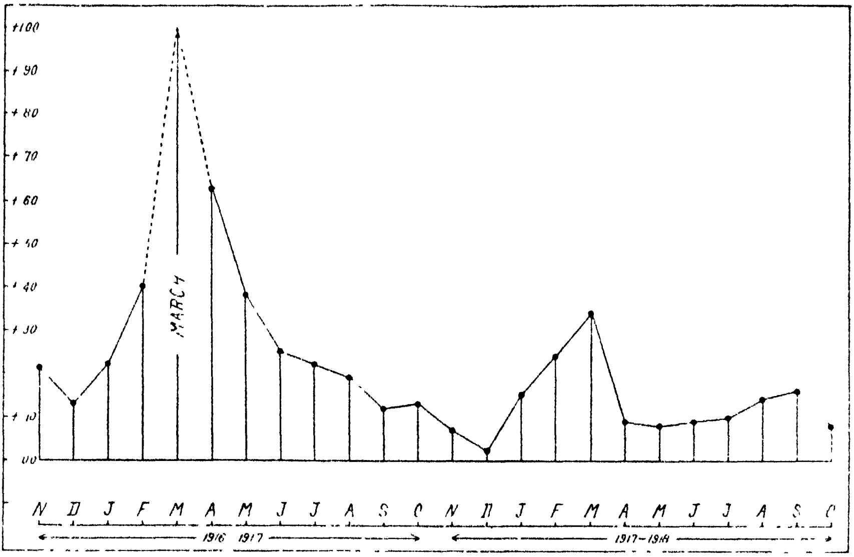

DIAGRAM 6.—Distribution of the magnitudes of the inter-mensual correlation coefficients for March of the first year.

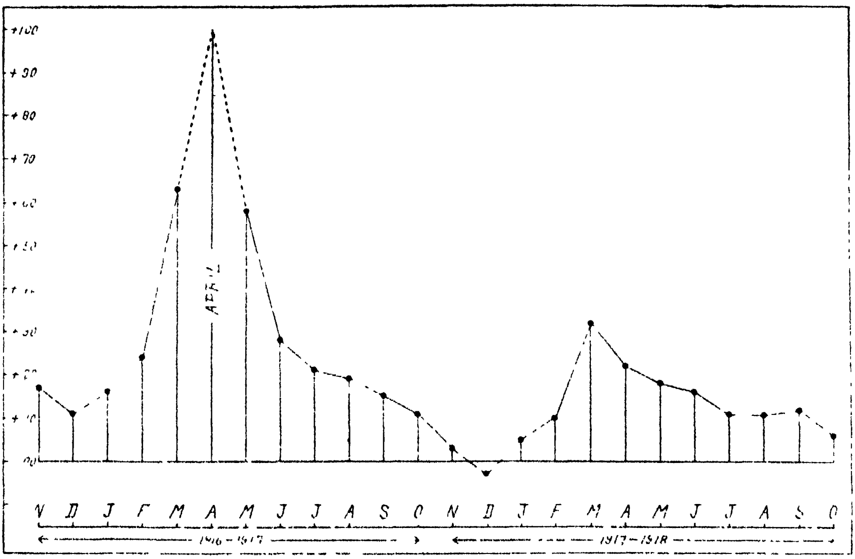

DIAGRAM 7.—Distribution of the magnitudes of the inter-mensual correlation coefficients for April of the first year.

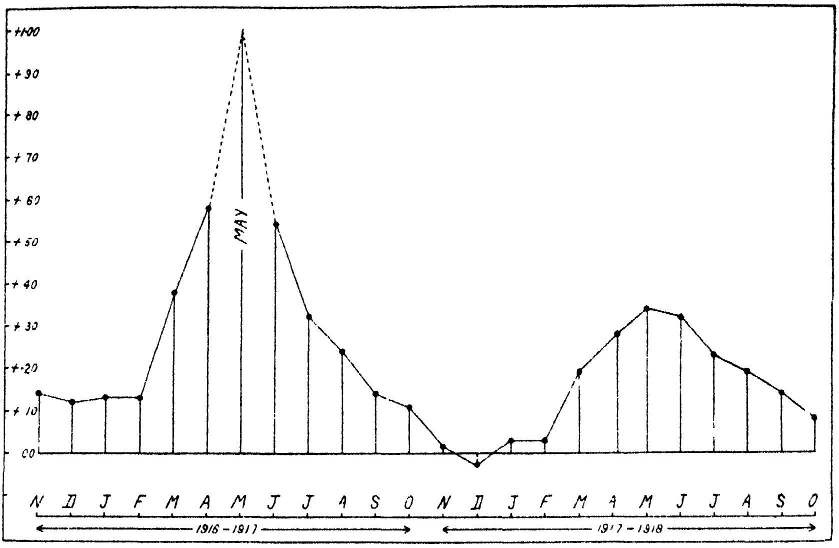

Diagram 8.—Distribution of the magnitudes of the inter-mensual correlation coefficients for May of the first year.

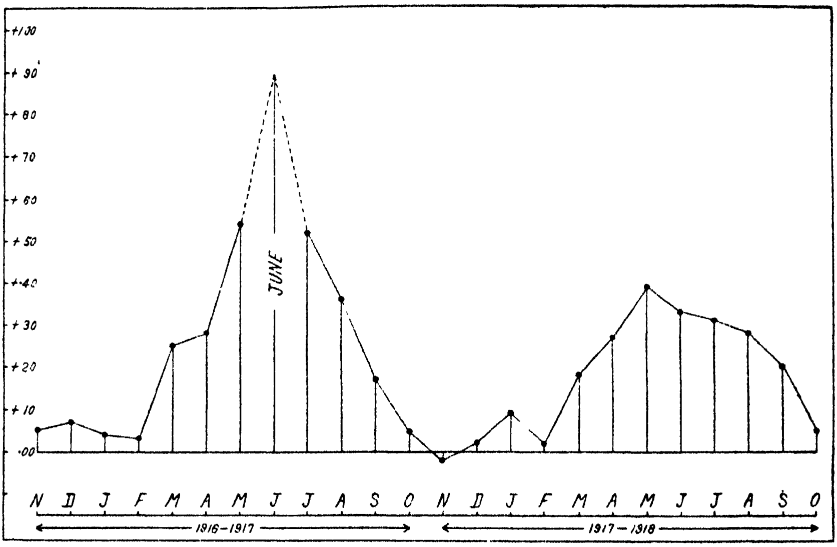

Diagram 9.—Distribution of the magnitudes of the inter-mensual correlation coefficients for June of the first year.

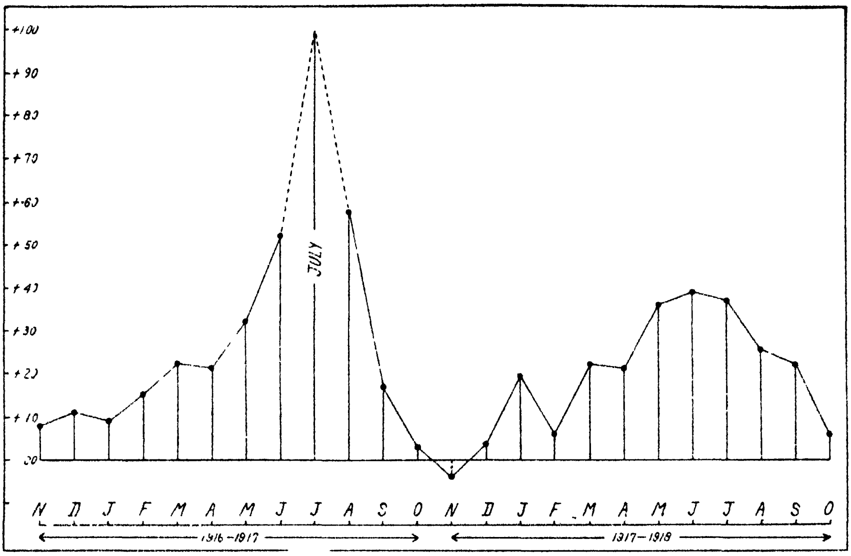

DIAGRAM 10.—Distribution of the magnitudes of the inter-mensual correlation coefficients for July of the first year.

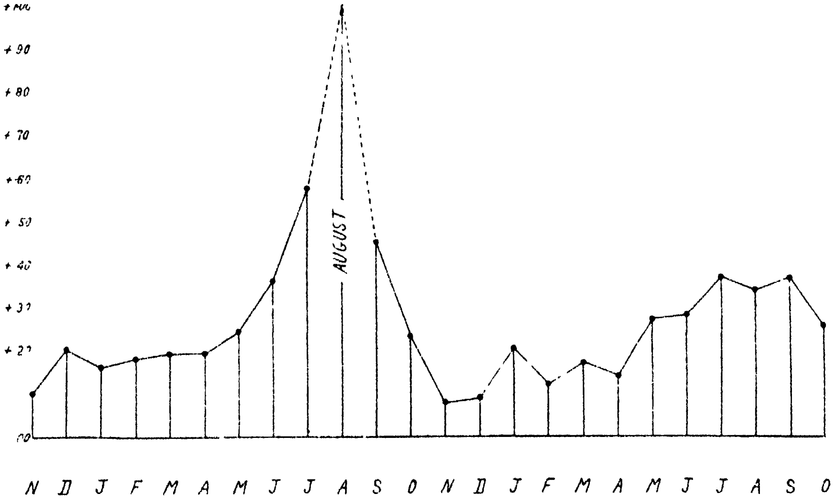

DIAGRAM 11.—Distribution of the magnitudes of the inter-mensual correlation coefficients for August of the first year.

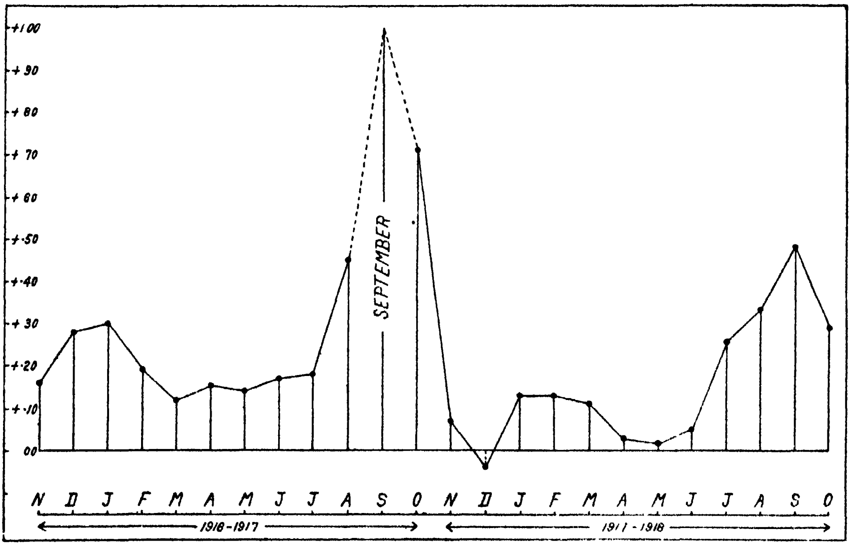

Diagram 12.—Distribution of the magnitudes of the inter-mensual correlation coefficients for September of the first year.

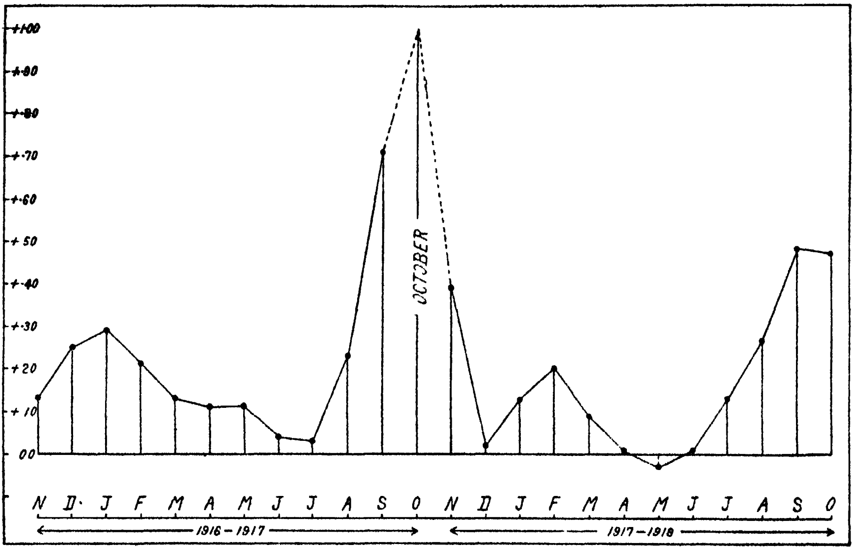

Diagram 13.—Distribution of the magnitudes of the inter-mensual correlation coefficients for October of the first year.

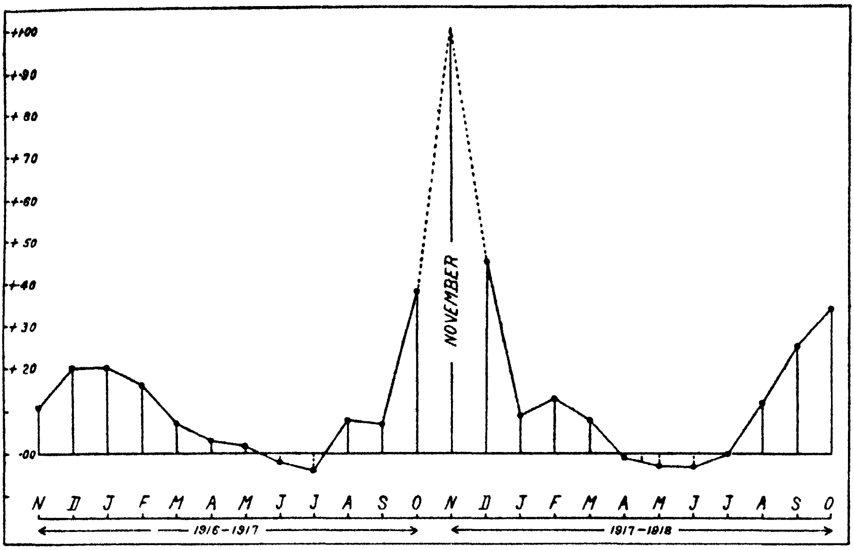

Diagram 14.—Distribution of the magnitudes of the inter-mensual correlation coefficients for November of the second year.

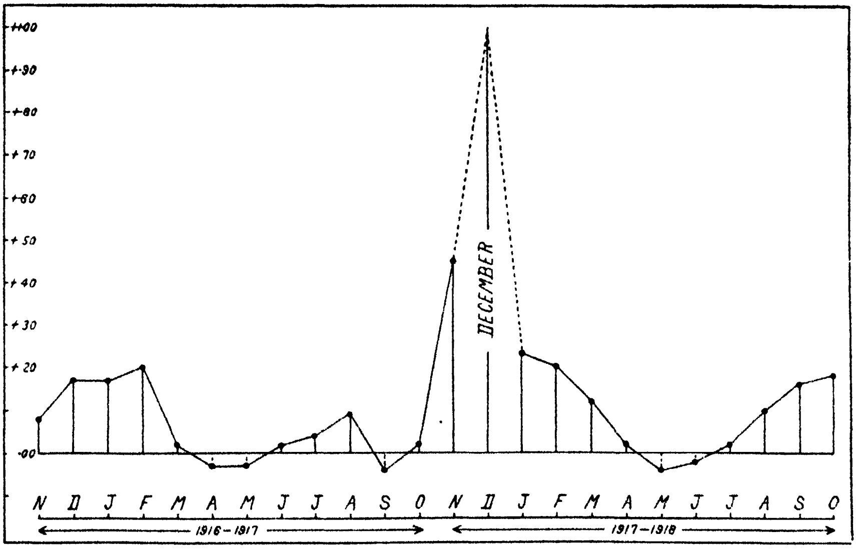

Diagram 15.—Distribution of the magnitudes of the inter-mensual correlation coefficients for December of the second year.

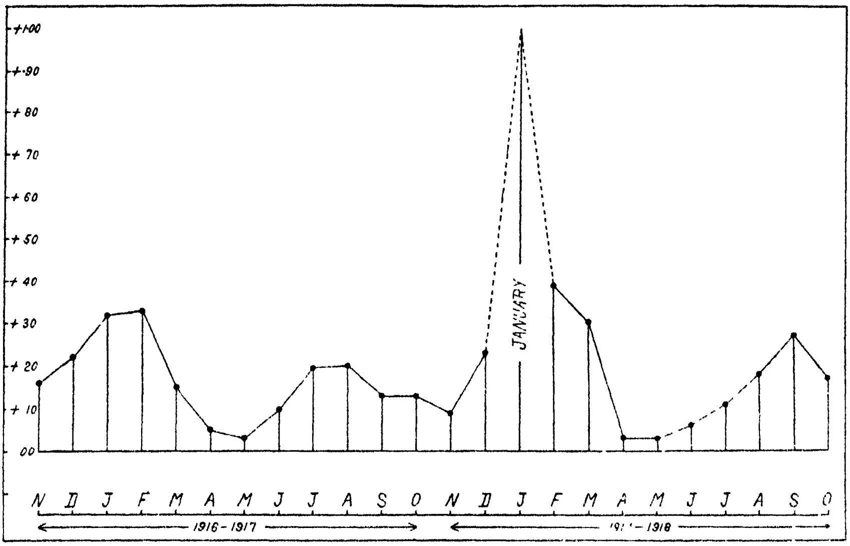

DIAGRAM 16.—Distribution of the magnitudes of the inter-mensual correlation coefficients for January of the second year.

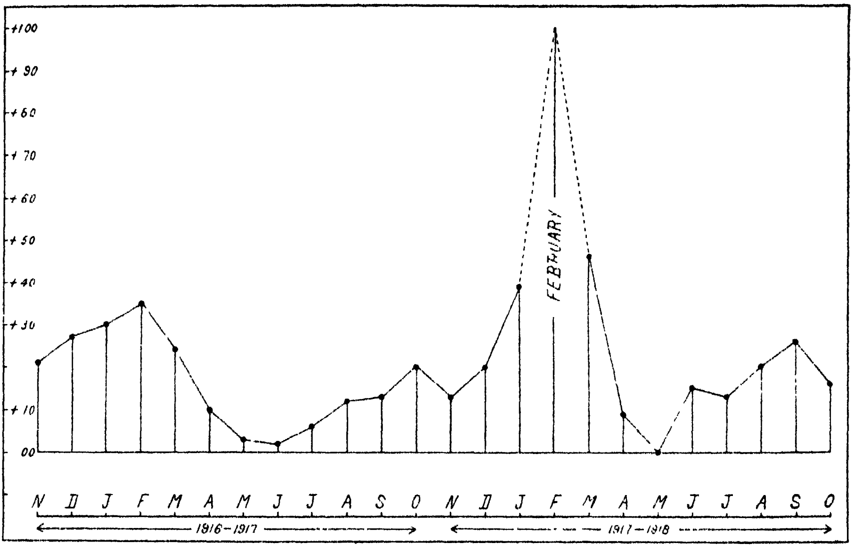

DIAGRAM 17.—Distribution of the magnitudes of the inter-mensual correlation coefficients for February of the second year.

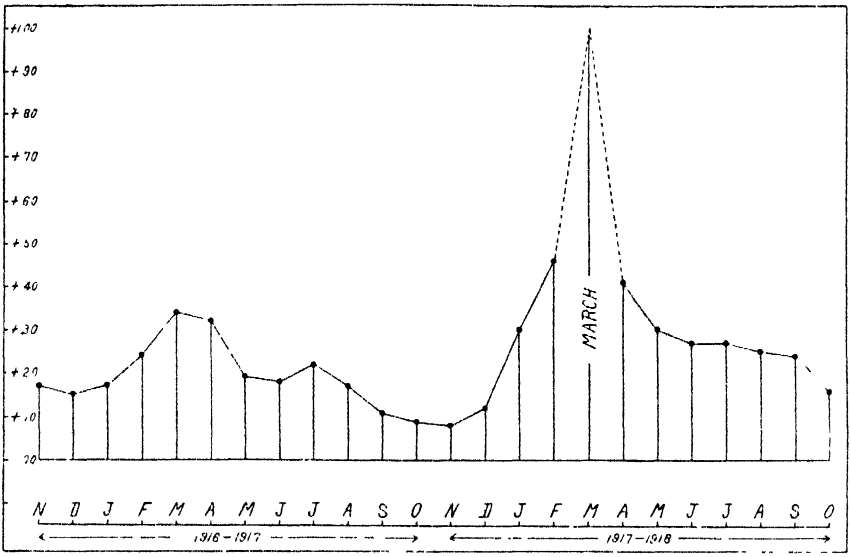

DIAGRAM 18.—Distribution of the magnitudes of the inter-mensual correlation coefficients for March of the second year.

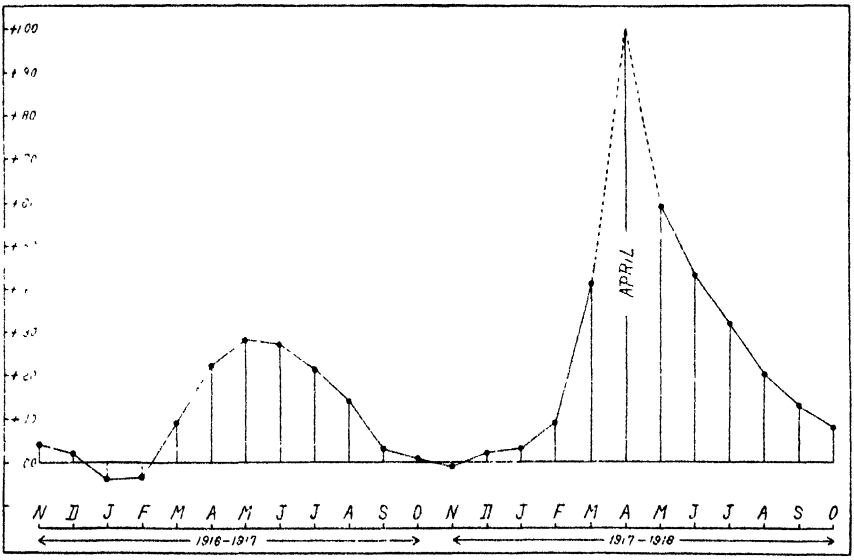

DIAGRAM 19.—Distribution of the magnitudes of the inter-mensual correlation coefficients for April of the second year.

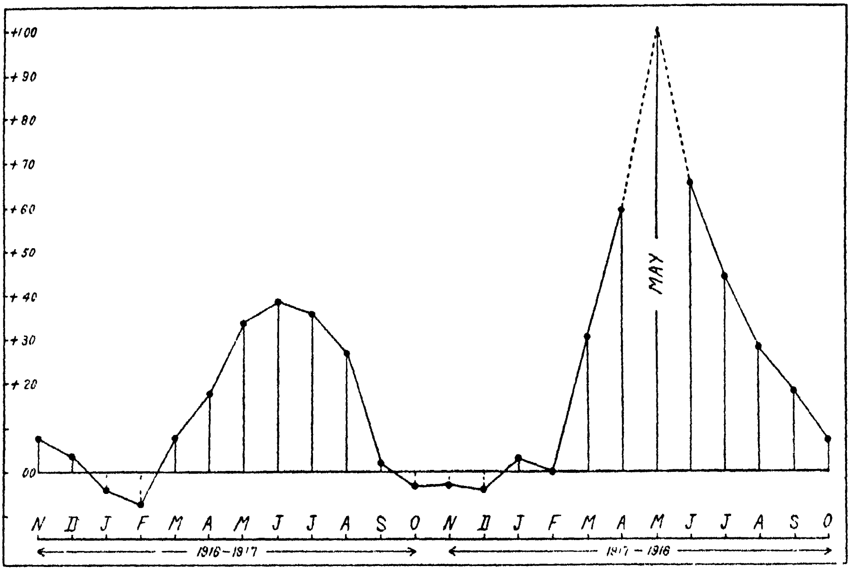

Diagram 20.—Distribution of the magnitudes of the inter-mensual correlation coefficients for May of the second year.

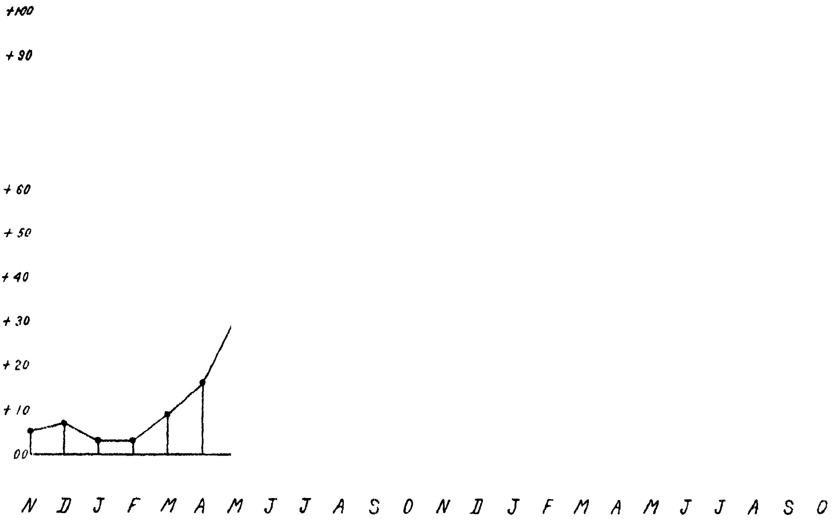

Diagram 21.—Distribution of the magnitudes of the inter-mensual correlation coefficients for June of the second year.

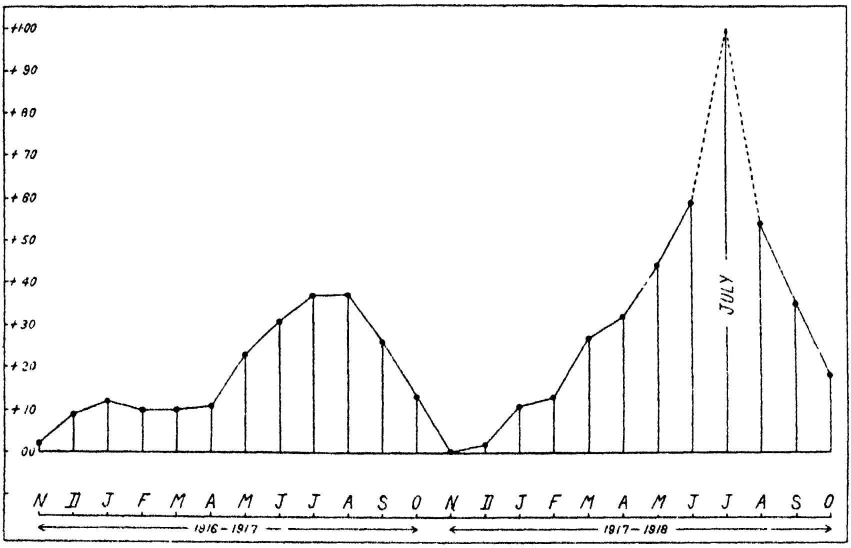

DIAGRAM 22.—Distribution of the magnitudes of the inter-mensual correlation coefficients for July of the second year.

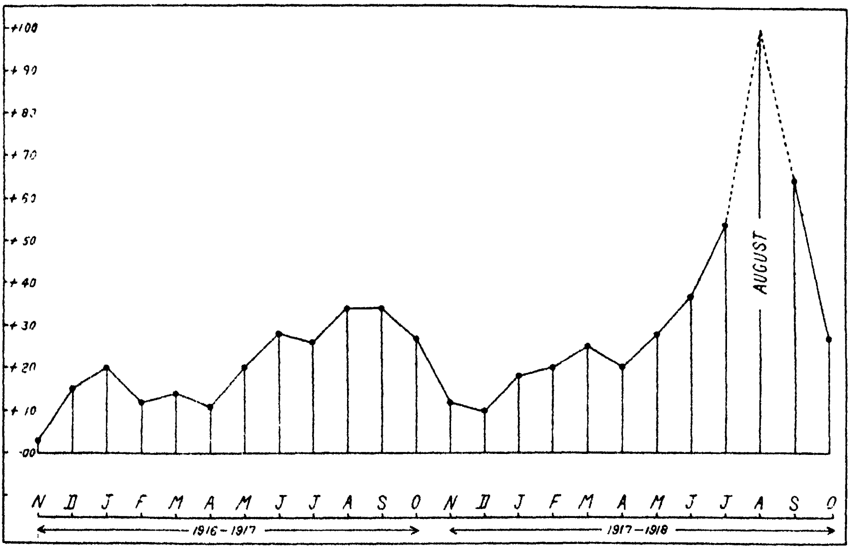

DIAGRAM 23.—Distribution of the magnitudes of the inter-mensual correlation coefficients for August of the second year.

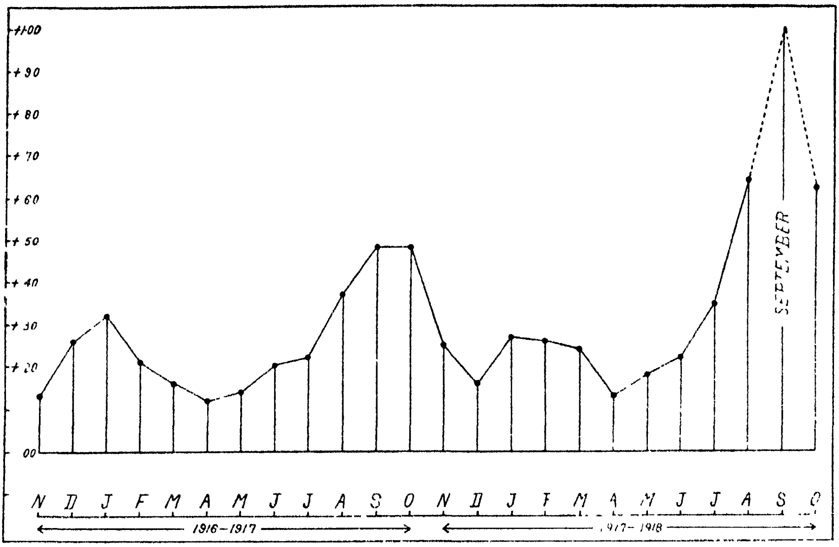

DIAGRAM 24.—Distribution of the magnitudes of the inter-mensual correlation coefficients for September of the second year.

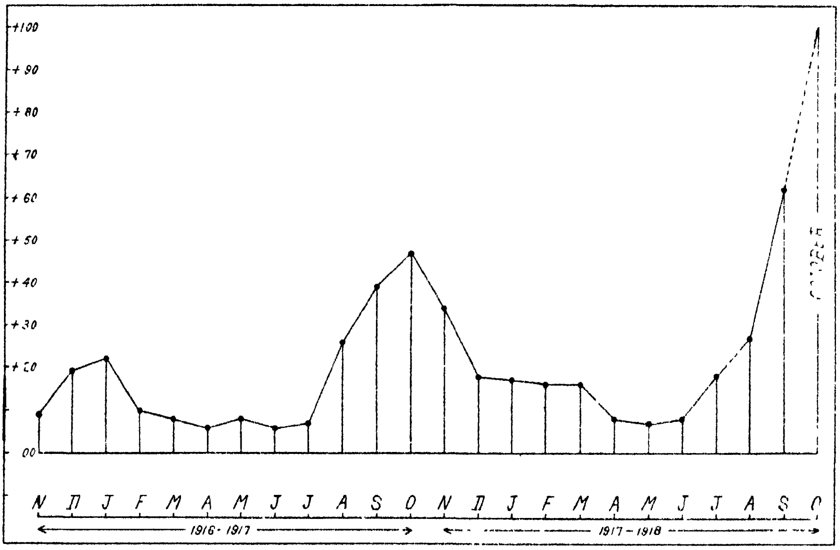

DIAGRAM 25.—Distribution of the magnitudes of the inter-mensual correlation coefficients for October of the second year.

The evidence shows in a wholly conclusive manner, therefore, that in general the intra-annual correlations, for both first and second year, are larger than the inter-annual correlations.

Turn now to the distribution of the ratios of the differences in correlation to their probable errors, as shown in table 7. We may unquestionably consider that differences which fall within the range of their probable errors are not significant. There are 36 such cases for the first year and 35 for the second. These may be disregarded for the purposes of the present discussion. If we consider that differences which are somewhat larger than their probable errors may in the long run be considered significant, we note that there are 89 negative differences as compared with 7 positive differences which are larger than their probable error in the first year. In the second year there are 79 negative as compared with 18 positive differences which may be considered statistically significant. If we regard as statistically significant only differences which are over twice as large as their probable errors, we note that there are 61 significant negative differences in the first year as compared with 3 which are positive. In the second year there are 54 which are negative as compared with 5 which are positive.

These tests of significance of differences fully confirm the conclusions drawn from the examination of the differences themselves, namely, that the intra-annual correlations for both the first and second year are sensibly higher than the inter-annual correlations for the first and second years.

The distribution of the magnitudes of the correlations between the months of the first two laying years

The problem of the distribution of the magnitudes of the correlation coefficients measuring the relationship between the productions of the different combinations of the twenty-four months for which records are available must now be considered.

After the calculation of the 276 correlation coefficients measuring the relationship between all the possible pairs of months in the first two laying years, our problem has been to secure some method of presentation which would bring out clearly the complex interrelationships under consideration.

The method adopted is a graphic one. The 23 coefficients measuring the possible correlations between each month and the 11 other months of the same year and the 12 months of the preceding or following year, as the case may be, are represented on one diagram in the following manner. The 24 months of the two years are distributed serially on the axis of abscissae. The egg productions of these months are regarded as a series of secondary variables. The correlation between the production of the month which is considered the primary variable with the production of each of these twenty-four months is represented on the scale of ordinates for each of the months. For the purposes of keeping the diagram for the 24 months an unbroken entirety and of furnishing a convenient standard of comparison, the correlation of the record of an individual month with itself has been properly considered unity. Such perfect correlation coefficients have been connected with the actually determined values showing the relationship between the record of the month under consideration (primary variable) and that of the other months (secondary variable) by broken lines.

Each of the diagrams represents the correlation between one of the 24 months under consideration and each of the other months of the two years. The month used as a basis of classification (primary variable) is indicated by the lettering on the ordinate. Such an arrangement of the correlation coefficients for a month and the other twenty-three months of the two years will for convenience be designated as a system of correlations for the month under consideration.

These diagrams were drawn up from table 4 of a former paper (Harris and Lewis 1922 a) which furnishes the inter-annual coefficients, and from table 1 of the present publication, which gives the intra-annual coefficients for the two years.

Diagrams 2 to 25 give the results for each month, arranged in chronological order from November of the first laying year to October of the second laying year.

A glance over the whole series of diagrams shows that the values of the correlation are practically without exception positive in sign. There are only a few cases in which the coefficients are negative. These are so small numerically that it is difficult, or impossible, to determine whether they actually denote a negative relationship between the months involved, or whether they are merely attributable to the errors of random sampling.

It will be convenient to discuss the systems for the individual months in a practically consecutive order.

The coefficient showing the relationship between the November production of the first year and the production of the subsequent months decreases rapidly from December to June of the first year. It then increases again to attain at least apparent submaxima[4] in September of the first and February of the second year. The minimum values are on the months of June of the first year and July of the second year.

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[4] Since the values shown on the charts represent the magnitudes of individual correlation coefficients and not frequencies, it is hardly proper to use the term mode, which is usually employed in the description of frequency distributions, to designate the larger magnitudes. We have, therefore, used the terms maximum and minimum to indicate the highest and the lowest values and submaximum to indicate values which are larger than the general run of constants.

The results for December of the first year are in general similar to those for November. The empirical minima are on June of the first year and April of the second year. The submaxima are on September of the first year and on February and September of the second year.

The correlation between the production of January of the first year and that of the antecedent and subsequent months shows a decrease in the magnitude of the coefficients measuring the relationship between January production and that of both antecedent and subsequent months as these months become more widely distant in time. Thus the correlation between the January and November production of the first year is materially smaller than that between the January and December production. Similarly, the correlations between January production on the one hand and March, April, May and June production on the other are much smaller than that between January and February production. The minimum values are found on June of the first year and on April and May of the second year. The submaxima are on September or October of the first and on January or February and September of the second year.

The system of correlations for February has a general similarity to that for December. Details may be studied from the diagram. We shall return to a further consideration of the secondary maxima noted in these systems later.

The systems of correlations for March and April of the first year are in general agreement. In both, the minimum value of the coefficient is found on December of the second year. In both, the distribution of magnitudes is bimaximal with the maximum on the months immediately preceding and immediately following the month used as a basis of classification, and with the submaximum on March of the second year.

This result suggests the existence of a hitherto unrecognized law underlying the interrelationship between the productions of the various months of the first two years, namely that *the maximum value of the correlation between widely separated months tends to be that for the combination of homologous months of the first and second years*. Possibly the law may be ultimately made more general by replacing "of the first and second years" by "of successive years."

The suggestion of the existence of this law finds ample confirmation in the results for May, June, July and August of the first year. For each of these four months the distribution of the inter-mensual correlation coefficients is conspicuously bimaximal. The minimum value for the system occurs on November or December of the second year in each of the four months. The submaximum occurs on either the homologous month of the second year or on a contiguous month. In the case of the system of correlations for May of the first year, the submaximum is on May of the second year. In the case of the system of correlations for June of the first year, the submaximum is on May of the second year, but the correlation for June of the first year and June of the second year is so nearly the same as that for June of the first year and May of the second year that they cannot be asserted to differ significantly. The difference between the two values is only 1.51 times as large as its probable error. In the case of the system of correlations for July, the submaximum is on June of the second year, but the coefficients for July of the first year and May, June and July of the second year are so nearly identical that the slight numerical differences may be due to the errors of random sampling. Finally in the system of correlations for August, the submaximum is on July, August or September of the second year. The value of the correlation for August of the first year and August of the second year is insignificantly smaller than either that for August of the first year and July of the second year or August of the first year and September of the second year.

The systems of correlations for September and October of the first year also confirm the conclusions to be drawn from those for March to August of the first year in so far as the law of the existence of the maximum value of the correlation for widely separated periods on the combination of homologous months is concerned.

The system for September has a conspicuous submaximum on September for the second year. The system for October has a conspicuous submaximum on September or October of the second year; the two coefficients are sensibly identical within the limits of the probable errors of random sampling. The distributions of the coefficients for these two months are sensibly identical.

In these months, however, there appears evidence of the true significance for a wider life cycle of the organism of one of the two laws already recognized on the basis of data for single years only. It has been noted

(HARRIS, BLAKESLEE and KIRKPATRICK 1918, p. 56-69) that

> " . . . . there is a more intimate correlation between the autumn and winter months at the beginning and end of the contest year than between the production of these months and the production of the spring and summer months."

The correlations for September and October show a *tendency for the occurrence of secondary maxima on both the earliest months of the first year and the latest months of the second year.*

These results, taken in connection with those for November, December, January and February of the first year, *confirm for the first year* previous conclusions concerning the existence of the law of more intimate correlation between the records of the autumn and winter months at the beginning and end of the contest year than between the records of these months and those of the intervening months. The present result *extends this law to the months of the second year. Previous conclusions are, therefore, fully verified as far as the records of individual years, considered independently, are concerned.*

When we consider the entire systems of correlations for September and October of the first year, instead of limiting attention to the relationships between the production of a single year, the existence of a trimaximal distribution for these months is clearly evident. The system of correlations for November of the second year is also conspicuously trimaximal. That for December is more irregularly so. The results for these two months (November and December of the second year), taken in conjunction with those for September and October of the first year, fully confirm the second law as already stated, and limited, and show that it is applicable to both first- and second-year production.

The two-year distributions of correlations suggest, however, that the greater correlation between the autumn and winter months of the individual years may be merely a phase of a more general law. This possibility must be borne in mind in examining the following systems.

The systems of correlations for January to October of the second year may first be considered with respect to the correlations between these months and subsequent months. The systems for January and February show a submaximum on September of the second year. This submaximum disappears in the systems for March to August of the second year, which, in the correlations with subsequent months, merely exemplify the law of decreasing correlation with increasing separation of the periods of time for which the correlations are determined. It is obviously impossible to test the applicability of this law of decreasing intensity of correlation with increasing separation of the two variables in time as far as subsequent months is concerned on the basis of the system for September of the second year, which has only one subsequent month, or on the basis of that for October of the second year which is the final month of the series.

Turning now to the systems of correlations measuring the relationship between the productions of March to October of the second year and the productions of antecedent months in the second and in the first year, we may note the following facts regarding the distributions.

In all eight months, the correlation with successively earlier months decreases in magnitude for from three to eight months at a varying rate but with some regularity in each case. It then increases to one or more secondary maxima. The distribution is conspicuously bimaximal in the systems for March, April, May, June and July (all of the second year). In the system for March of the second year, the submaximum is on March of the first year. In the system for April of the second year, the first year maximum is on May, but the magnitude for April of the second year and April and June of the first year are sensibly the same as that for April of the second year and May of the first year. In the system for May of the second year the maximum correlation for the first year is that for June, but the coefficients for May of the second year and May, June and July of the first year are practically the same. In the system for June of the second year, the first-year maximum (submaximum of the system) is on July. In the system for July of the second year, the first-year maximum is on July or August, which have coefficients of almost exactly the same magnitude.

The distributions of magnitudes for these five months of the second year confirm in a striking manner, therefore, the results for the occurrence of a submaximum on the homologous month of the second year noted above for the systems for May, June, July and August of the first year.

· Bimaximality is not so sharply marked in the systems for August, September, and October of the second year. In the systems for these months there is a rather compelling suggestion of a tertiary maximum which is on January in all the systems. Leaving this tertiary maximum out of account for the moment, we may note that in the system for August of the second year, the maximum for the first year is on the sensibly identical coefficients, August or September. In the system for September of the second year, the maximum for the first year is on the sensibly identical coefficients for September of the second year on the one hand and September or October of the first year on the other. Finally, in the system for October of the second year, the maximum for the preceding year is conspicuously on October.

Turning back to the systems for December, January and February of the first year and examining them in the light of the systems for the final months of the second year, we may see evidences for a tertiary peak on the final months of the second year in these systems. In the systems for December, January and February, it is on September of the following year. It is so clearly marked in these cases that there can be little doubt of the reality of its existence.

Inspection of the systems for November and March of the first year will detect suggestions of tertiary peaks on September of the second year.

SUMMARY DISCUSSION AND CONCLUSIONS

The purpose of this paper, which is one of a series dealing with the various problems of the physiology of egg production in the domestic fowl, is to consider the magnitudes and the distribution of the magnitudes of the correlation coefficients measuring the relationship between the egg productions of the individual months of the first two laying years.

We have designated the correlation between the records of any two months of the bird's life as an inter-mensual correlation coefficient. The inter-mensual coefficients which involve the months of the same laying year, either first or second, have been designated as intra-annual coefficients. Those involving months belonging to different laying years have been designated as inter-annual coefficients.

We have designated the whole series of inter-mensual correlations for the first year or for the second year as a system of correlation coefficients. Thus, we refer to the intra-annual system for the first year, and to the intra-annual system for the second year. Similarly, we designate the whole series of inter-mensual correlation coefficients involving the months of the two different years as an inter-annual system for the first and second year. In the analysis of these systems it has been necessary to consider the distribution of the magnitudes of the coefficients between each individual month of the series (considered a primary variable) and each other month of the series (considered a secondary variable). Such a series of coefficients has been called a minor system, or merely a system when no ambiguity can result from such abbreviation. Such minor systems are designated by the month considered as the primary variable in limiting the minor system.

The complete records for the first two laying years for 443 White Leghorn birds studied at the first INTERNATIONAL EGG LAYING AND BREEDING CONTEST at Vineland, New Jersey, makes possible an investigation of the magnitudes of the $\frac{1}{2}(24\times23)=276$ correlation coefficients measuring the relationship between the months of the first two years of the White Leghorn fowl.

While the intra-annual correlation coefficients for the first year have been published for a number of series of birds, and while the inter-annual coefficients for the Vineland series are available (table 4 of HARRIS and LEWIS 1922b) those for the second year have not, heretofore, been determined.

The results show that the intra-annual coefficients measuring the closeness of interdependence of the productions of the various months of the second year are of about the same average value as those of the first-year records of the same series of birds. The average value of the system of intra-annual (inter-mensual) correlations for the first year is 0.2390, whereas that for the system for the second year is 0.2199. The difference between the two systems of coefficients is 0.0191 ± .0093.

The intra-annual correlation coefficients measuring the relationship between the productions of the months of the same year are not merely similar in average magnitude, but the distribution of the magnitudes of the inter-mensual coefficients for each of the individual months of the second year bears a close similarity to the distribution of the homologous coefficients for the months of the first year. This may be shown by comparing the right half of diagram 14 with the left half of diagram 2, the right half of diagram 15 with the left half of diagram 3, and so on to the end of the series, where the right half of diagram 25 is compared with the left half of diagram 13.

The general consistency of the inter-mensual correlation coefficients for the first and second year is measured by a coefficient of correlation between the two systems of 0.7839 ± .0320. Thus there is a high but by no means perfect correlation between the two systems. Biological explanations for certain of the discrepancies between the coefficients for individual combinations of months are suggested.

The investigation of the distribution of the magnitudes of the inter-mensual correlation coefficients throughout the entire biennial period under consideration is a logical sequel to earlier researches. It has been shown by NIXON (1912), by BALL and ALDER (1917), and by HARRIS and LEWIS (1922 b), that in the White Leghorn at least, there is a material correlation between the egg records of the first two years. In an earlier paper we have shown that this correlation is so close that on the average the production of each individual month of the second year is correlated with the production of each individual month of the first year. In other papers it has been shown that in the White Leghorn, Rhode Island Red and White Wyandotte breeds the distribution of the inter-mensual correlations for the first year is an orderly one, such that it is possible to express the distributions of magnitude of inter-mensual correlation in two laws.

Since a number of studies on the relationship between the production of the first two years of the bird's life indicate that these records are correlated, while other series of studies show that the inter-mensual coefficients for the first year are distributed in a lawful manner, it seems quite reasonable to assume that the first two years of the bird's life may be regarded as a unit and to enquire concerning the nature of the distribution of the magnitudes of the inter-mensual correlations for this whole period of time.

The results show that when the correlations for the individual years are considered independently, previous conclusions concerning the distribution of the magnitudes of the correlation coefficient measuring the relationships between the production of individual months are substantiated. When these coefficients are considered for a period of two years, however, it appears that the two laws heretofore stated are merely phases of a more general law, namely, that the correlation between any month considered as a primary variable and subsequent or antecedent months considered as secondary variables, first decreases rapidly as the primary and secondary variables become more widely separated, but again increases as the secondary variable approaches a homologous period of time in another year, after which it again decreases. There is, therefore, a rhythmic or periodic change in the magnitudes of the correlation coefficients as primary and secondary variables become more widely separated in time.

While these conclusions are based upon one series of data only, the consistency of the results evidences for their validity.[5] While the present results pertain to the White Leghorn breed only, the fact that other breeds have been found in other studies to obey the two laws originally formulated for the distribution of the correlation coefficients of a single year, justifies the assumption that the law here stated may be found to be applicable to them also.

The explanation of this phenomenon requires more intensive biological and statistical investigation. Its definiteness indicates clearly the importance of such research.

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[5] It is proper to state that analysis of another series of data for the White Leghorn breed does not give results so consistent as those published here, but evidences of untrustworthiness in the second series of data which became known to us only after the analysis was undertaken leads us to feel that they should be discarded.

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MEIOSIS IN THE POLLEN MOTHER CELLS OF *OENOTHERA BIENNIS* AND *OENOTHERA BIENNIS SULFUREA*

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INTRODUCTION

The material upon which this study is based was obtained during the summer of 1920 in the experimental garden of Professor B. M. DAVIS, at Ann Arbor, Michigan. The culture of *Oe. biennis* L. from which fixations were made was the ninth generation in a selfed line descended from seeds obtained originally from Professor HUGO DE VRIES, the strain being called by Professor DAVIS, *biennis* H. It is not to be confused with the *Oe. biennis* studied cytologically by Professor DAVIS, which is a different species derived from American sources (DAVIS 1910). Material of *Oe. biennis sulfurea* was taken from a culture which represented the fourth generation in a selfed line from seed furnished by Professor DE VRIES.

Six extensive fixations of *Oe. biennis* were made from two plants; and five fixations of *Oe. biennis sulfurea* were secured from five individuals. The critical periods of "diakinesis" and the heterotypic metaphase, as well as all other stages, were plentifully obtained from both plants of *biennis*. I missed both of these important phases, however, in all but one of the fixations of *biennis sulfurea*; but this one fixation has yielded material clearly indicating that in cytological behavior this variety is in all respects similar to *Oe. biennis*.[1]

As fixing solutions, weak Flemming with maltose and urea added, and one of ALLEN'S modifications of Bouin's solution were used. The former failed in large measure, probably because of the waxy layer which coats the anthers, but the fixations obtained with the latter were in general satisfactory, and often excellent. For the formula of this fluid see CLELAND 1924.

DESCRIPTION

Early prophase stages

I do not propose to devote much space to a discussion of the earlier stages of meiosis in these forms, inasmuch as they appear not to differ from corresponding stages in species already described (CLELAND 1922, 1924). I have made a careful study of cells in the archesporial stage and in early prophase, especially in *Oe. biennis*. In general, as well as in particulars, the history conforms closely to that found in *Oe. franciscana* and *Oe. franciscana sulfurea*, and can be summarized briefly as follows:

1. Archesporial cells in the resting stage prior to the beginning of the heterotypic prophase, are characterized (a) by a copious network of delicate threads, which show very little indication of parallel arrangements; and (b) by the presence, usually, of one large nucleolus and several distinctly smaller ones.
2. The process which takes place during early prophase stages may be described as a gradual transformation of the fine-meshed network of delicate threads into a large-meshed system of much coarser threads. This is apparently accompanied by the contraction of certain threads, and their absorption into the body of others, a process that gives rise to unequal stresses and strains within the system, which may help to cause its collapse toward one side of the nucleus, resulting in the stage of synizesis.

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[1] In an interesting paper by STERLING H. EMERSON (1924), that has just come to hand, figures are given of the "diakinesis" stage in *Oe. biennis* and *Oe. biennis sulfurea*, clearly showing in both plants the presence of circles, such as are to be described in this paper. Thus, the evidence for the existence of circles composed of chromosomes arranged end to end rests not alone on the few plants examined by me, but upon additional material collected from the same strains, but at a later date. In regard to chromosome behavior during the heterotypic metaphase, however, EMERSON takes exception to the results described in this paper.

3. It is inevitable that such a procedure should result in occasional, even frequent, cases of parallelism of threads, but these are no more numerous or extensive than would be expected in view of the process, and can hardly be considered as evidence of a synaptic conjugation of homologous elements.

4. In these forms, as in those previously studied, the synizetic knot generally lies next to the side of the nucleus which was in all probability first reached by the fixing fluid. This, to my mind, casts suspicion upon the nature and cause of synizesis, and suggests that the phenomenon may be an artifact. The various contractions and adjustments going on in the reticulum at this time may cause it to become torn away from the nuclear membrane, and thus render it easy for even the best fixing fluid to change its position, or even to accentuate the amount of shrinkage. It is no doubt more difficult to fix without shrinkage a thread whose ends are free, than one whose ends are attached to some more or less stable object.

5. The nucleoli are in intimate contact with the reticulum during the resting stage and the heterotypic prophase, and it seems fairly certain that during synizesis material is drawn from these bodies and added to the substance of the network. This is indicated first by the fact that the endonucleolus does not appear until late synizesis, being apparently masked at earlier stages. It is further suggested by the fact that the threads which connect the nucleolus with the reticulum are as a rule much swollen during late synizesis, as though gorged with the outpoured material. Conditions such as are depicted in the paper on *franciscana sulfurea* (CLELAND 1924, figure 3) are occasionally seen, though such striking figures are not common.

6. It is an interesting fact that during the stages when the endonucleolus is apt to appear for the first time, this little body may frequently be observed to have an organic connection with a certain portion of the reticulum (plate 1, figure 1. See also CLELAND 1922, figures 14, 16; 1924, figure 4). I am not at present prepared, however, to suggest what may be the significance of this fact.

The open spireme

Many cases have been seen in which the reticulum, after emerging from synizesis, has become quite evenly distributed throughout the nucleus, so that the individual parts may be easily examined (plate 1, figure 1). In comparison with the total number of cells studied, however, those presenting this picture clearly are quite rare, and it is either a stage very

EXPLANATION OF PLATES

All figures were drawn with the aid of a camera lucida, using a Spencer compound binocular microscope, and a Bausch and Lomb 2mm apochromatic objective with 25x compensating ocular. They have been reduced one-fourth in reproduction; present magnification approximately 2800 diameters.

All figures represent *Oe. biennis,* except figures 12 and 13, which were drawn from *Oe. biennis sulfurea.*

PLATE 1

FIGURE 1.—The open spireme stage. Threads are single. The endonucleolus is in contact with the reticulum.

FIGURE 2.—Very early second contraction, the threads in the center becoming greatly swollen, those at the periphery being thrown into radiating loops. Note the close approximation of the sides of some of the loops.

FIGURES 3, 4, 5.—More advanced stages in second contraction.

FIGURE 6.—Late second contraction, perhaps beginning to unfold. Individual chromosomes are becoming recognizable.

FIGURE 7.—The unfolding process nearly completed, many of the individual chromosomes clearly visible.

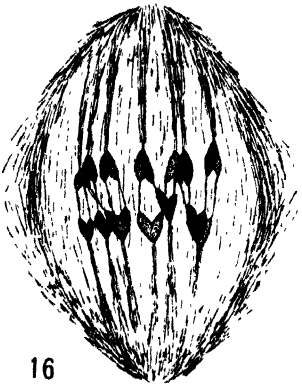
FIGURES 8, 9, 10.—"Diakinesis," showing the circle of 8 and the circle of 6 linked together.

FIGURE 11.—The circles have fused to form a circle of 14 chromosomes. The only undoubted case of this observed.

FIGURES 12, 13.—"Diakinesis" in *Oe. biennis sulfurea,* showing the circle of 8 and the circle of 6. In figure 12 they are linked, in figure 13 they have separated.

FIGURES 14, 15.—The multipolar-spindle stage with the circles intact. The spindle fibers have been omitted.

CLELAND, R. E., MEIOSIS IN *Oenothera biennis* AND *Oe. biennis sulfurea*. PLATE 1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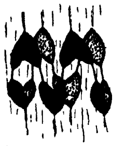
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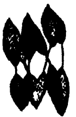
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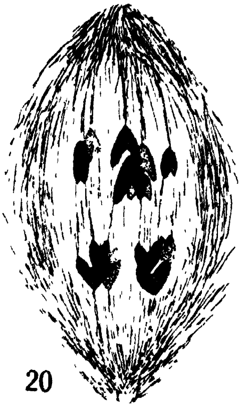
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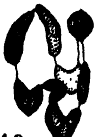
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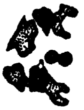
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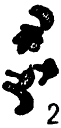
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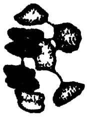
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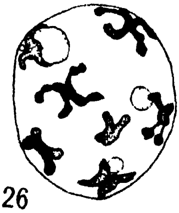
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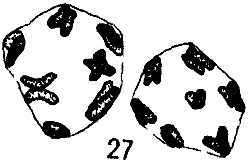
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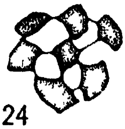
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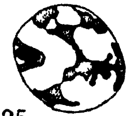
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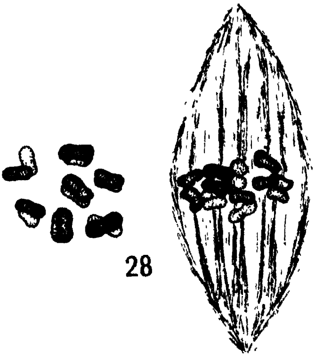
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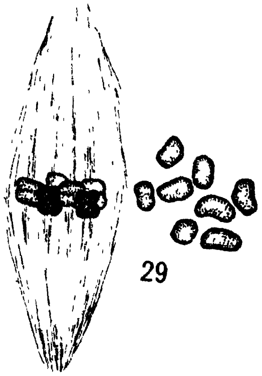
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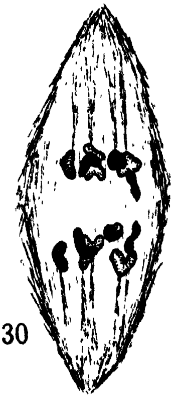
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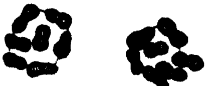
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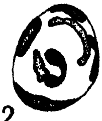
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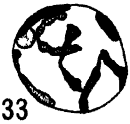
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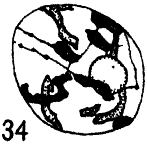
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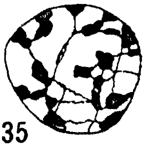
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quickly passed through, or else in many cells the threads fail to become widely spread, and remain instead in a more or less dense tangle in one part of the nucleus. During this period, one may look in vain for indications of a split in the threads, or of close approximation. The open spireme stage affords very little evidence, therefore, for the presence of parasynapsis.

The threads constituting the open spireme are fairly uniform in diameter throughout the nucleus, and at times appear quite smooth, while at other times there is a more or less marked suggestion of chromomeres. Free ends are rarely found, and are probably normally not present. The system at this time is still in the form of a reticulum, but the meshes are few in number, and quite extensive in size. The large nucleolus continues, as a rule, to maintain a lateral position, but loses its flattened appearance, and once more approximates the original spherical shape. The endonucleolus remains in contact with a part of the spireme for a varying length of time, the connection being broken in some cells before the open spireme stage is formed, and in others not until later.

Second contraction

After a time, the threads begin to lose their evenness of diameter. Those in one portion of the nucleus, usually toward the center, or occasionally near the largest nucleolus, begin to thicken, apparently at the expense of the ones nearer the periphery (plate 1, figures 2 and 3). It becomes evident with the continuance of this process that the peripheral threads are arranged into loops which radiate out in all directions. As second contraction proceeds, the threads in the central portion become thicker and more massed, while the loops gradually contract and are little by little drawn in toward the ever enlarging central region (figures 3 and 4). During this process of contraction, the two sides of individual loops often become closely applied. They are so closely appressed in some cases as almost to give the impression of a single thread, double in thickness (figure 5). All transitions may be noted, however, between this extreme and cases where the loops are very broad.

The loops, gradually shortening, and often losing much of their stainable material, are usually visible until a very late stage,—in fact, it may be rather confidentially asserted that no stage in second contraction has failed to show them. While in a good many cells, at the time of maximum contraction, they have become almost, if not entirely, withdrawn into the central mass, there are nevertheless many other cells which seem to suggest that they need not necessarily entirely disappear. There is in fact no sure way of telling whether in certain cells the contraction is still going on, whether it is still at its maximum, or whether the cell is beginning to emerge from this condition. We apparently find radiating loops in all stages. They are comparatively thin in diameter in early contraction stages, become gradually thicker during the time of maximum contraction, and when they emerge are noticeably thicker than they were before. There is little doubt in my mind that the loops which emerge from the second contraction stage, as illustrated in plate 1, figures 6 and 7, are the same as those which entered it in the first place (figures 3 and 4). This is evidenced not only by the presence of loops in all stages of second contraction, but also by the fact that during this period one may easily find incipient stages in the differentiation of the individual chromosomes which are soon to make their appearance. Notice, for instance, the loop in the upper left-hand corner in figure 4, and the evident suggestion of individual chromosomes in figures 5 and 6. Further negative evidence is afforded by the lack of indications of a transverse separation at the ends of the loops which enter the contraction stage, and the subsequent longitudinal splitting of the separated halves of these to form the loops which finally emerge. Altogether, I feel that it is almost certain that the spiremes which finally appear in "diakinesis," and which are unquestionably univalent in character, can be traced backward through the second contraction stage by means of the radiating loops, and can be shown to be the same as the open spireme, much shortened and thickened and simplified, but nevertheless the same. The open spireme is without doubt univalent in character, and not bivalent.

One further word should be added with regard to this stage. At or near the time of maximum contraction, one sometimes notes that the sides of the radiating loops appear to be twisted around each other (figure 5). This approach to a strepsinema condition might result occasionally in the kind of chromatin exchange known as crossing over.

"*Diakinesis*"

When the second contraction knot begins to unfold, it soon becomes evident that the elements which are emerging are not in the nature of paired univalents, as they are in most plants. Instead, we find that in both *biennis* and *biennis sulfurea* there are two spiremes in each nucleus and that each spireme is in the form of a closed circle. One circle is composed of 6 univalent chromosomes attached end to end; the other o 8 chromosomes; and the two circles are usually found linked together (figures 8 to 10, 12, 13). Naturally enough, the linked circles are often in physical contact with each other at one or more places, and it sometimes happens that contact occurs in such a way that a thread connecting two chromosomes in one circle lies against the connection between two chromosomes of the other circle. In such cases, the circles appear to be fused into a figure 8. An actual fusion of the two threads probably takes place occasionally, for I found one nucleus in *Oe. biennis* in which they had apparently fused and then separated at right angles to the plane in which they had come together, the result being the formation of a large circle of 14 chromosomes (figure 11). Excepting this one case, and the occasional instances in which the linked chromosomes simulate a figure 8, however, the arrangement in the stage just after second contraction is constant, and is one in which two circles, one composed of 6 and the other of 8 chromosomes, are closely linked together. There is therefore no pairing whatever between any of the homologous chromosomes of either plant during this stage, in marked contrast to what we normally expect in diakinesis in most plants.

During the period that follows, leading up to the heterotypic metaphase, certain changes occur in the nucleus. In the first place, the circles of chromosomes become, as a rule, unlinked. We also find that they sometimes break open. In many cases where I have observed open chains instead of circles, the break has been due to the section knife, but numerous instances have been seen in which this appearance could not have been due to any treatment accorded the material. The circles probably break open naturally at times, particularly when they are becoming unlinked. Their separation no doubt involves a temporary break in one of the circles. The broken ends fail to become reunited, and the spireme from that time on appears as a chain of 6 or 8 chromosomes, instead of a closed circle.

A description of such an unusual type of "diakinesis" should be backed by a considerable mass of evidence. I have records of 113 nuclei of *biennis* in which, at this stage, one or both of the spiremes were found to be complete. In 54 cases, both circles were closed and intact; in 21, the circle of 6 was intact, but the other circle was only partially present in the section, or complete but open, in the form of a chain; in 22 cases, the circle of 8 was intact, the circle of 6 either open or incomplete; in 7, the circle of 6 was complete and open, the circle of 8 incomplete or absent; and in 9, the circle of 8 was complete and open, the circle of 6 incomplete or absent. Corresponding figures for *biennis sulfurea* follow: In 14 cases, both circles were closed and intact; in 12 cases, both circles were complete, but one was open; in 3 cases, both were complete and both were open; in

10 cases, the circle of 6 was intact, the rest incomplete or obscure; in 5 cases, the circle of 8 was intact, the rest incomplete or obscure. In both forms, every nucleus that was sufficiently complete and clear to show any arrangement displayed the one just described. There can be no doubt, therefore, I believe, that the condition here described is constant for the species.

During the period of "diakinesis," we find that the chromosomes shrink greatly in size. When first they become visible, they are long and large (figure 8), but by the end of the period, they have become reduced to small, short, fat little bodies, all pretty much of a size, and not individually distinguishable (figures 14 and 15). There is bound to be a certain amount of unevenness in such a process of shrinkage. Some will contract a bit faster than others, perhaps one end of a chromosome somewhat faster than the other end. For a time it may look as though there is some recognizable difference in the size of the various chromosomes, but this difference soon disappears. The slight variations that can be detected during this period, therefore, seem to be neither constant nor significant.

The stage marks also the passing of the nucleolus. Plastered over against the nuclear membrane, and empty-looking except for a small black-staining endonucleolus, it begins to melt away, disappearing by insensible degrees.

The heterotypic metaphase and anaphase

When the chromosomes have ceased to shrink, and the nucleolus has disappeared, the nuclear membrane also dissolves away. Meanwhile, spindle fibers have made their appearance in the cytoplasm all around the nucleus, and these, as soon as the nuclear membrane has gone, penetrate to the interior, and surround and attach themselves to the chromosomes. At first, the spindle is multipolar, and the circles of chromosomes, still intact, lie without order or polarity (figures 14 and 15). Then the spindle becomes bipolar, and the circles begin to assume a definite position in the cell. I have already described this process in *Oe. franciscana sulfurea*. In the forms we are now considering, as in that one, the circles or chains do not as a rule break up at this time, but retain their identity throughout metaphase. The question at once arises as to whether it is possible for chromosomes, which come to the equatorial plate bound together into closed circles, to be subject to the laws of distribution which ordinarily prevail in organisms at this period. It is easy to see that chromosomes which are paired are almost certain to be separated to opposite poles in the heterotypic division, but when they are not paired, and all of them

EXPLANATION OF PLATE 2

FIGURE 16.—Heterotypic metaphase, showing the regular zigzag arrangement of the chromosomes. Both circles are intact.

FIGURE 17.—The circle of 8 in heterotypic metaphase, regularly zigzag.

FIGURE 18.—The circle of 6 in heterotypic metaphase, regularly zigzag.

FIGURE 19.—An irregularity in the zigzag arrangement in a circle of 8.

FIGURE 20.—Heterotypic anaphase. Indications of the zigzag arrangement still persist. This is much more striking in the original than it can be made in a drawing, where all chromosomes have to be shown in one plane.

FIGURES 21, 22.—Chromosomes in anaphase, showing an early longitudinal split. Spindle fibers omitted.

FIGURE 23.—Telophase with early indications of the longitudinal split.

FIGURE 24.—The more usual appearance in telophase, in which the chromosomes have not yet revealed their split nature.

FIGURE 25.—Early interkinesis.

FIGURE 26.—Mid-interkinesis, the chromosomes in the form of Maltese crosses. Chromosome individuality is clearly maintained.

FIGURE 27.—Late interkinesis, just before the disappearance of the nuclear membranes. Both nuclei in the cell are shown.

FIGURE 28.—The homoeotypic metaphase, showing both mitotic figures. The normal number of chromosomes present in each.

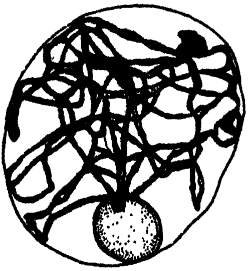
FIGURE 29.—The same. An unequal distribution of the chromosomes has occurred in the reduction division.

FIGURE 30.—The homoeotypic anaphase.

FIGURE 31.—Early telophase. Two of the four complexes shown.

FIGURES 32, 33, 34, 35.—The return of the granddaughter nucleus to the resting stage.

CLELAND, R. E., MEIOSIS IN *Oenothera biennis* AND *Oe. biennis sulfurea.* PLATE 2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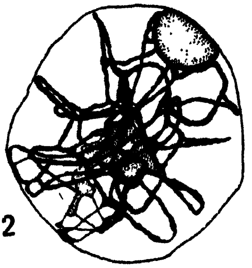
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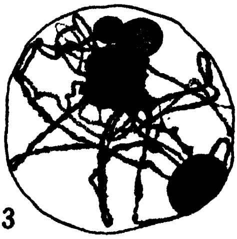
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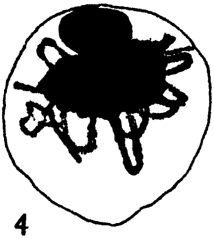
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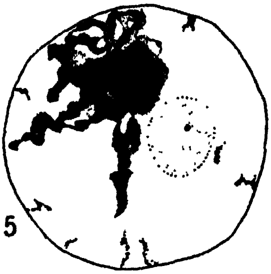
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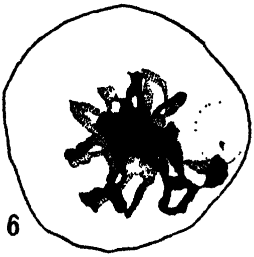
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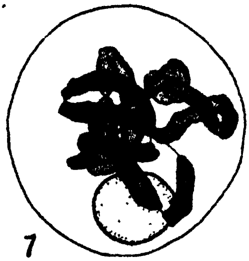
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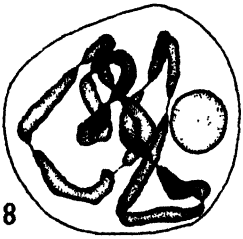
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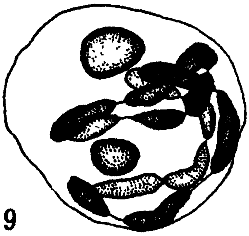
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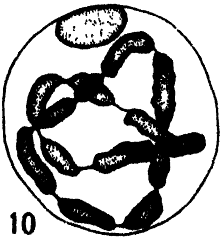
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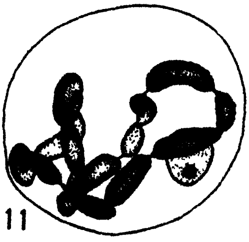
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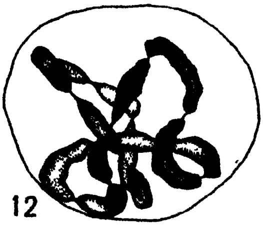
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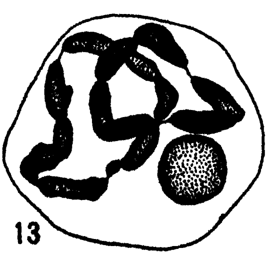
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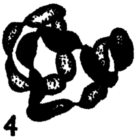
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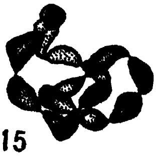
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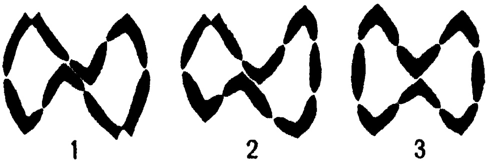
are more or less equally exposed to whatever influences may be at work between the equatorial region and the poles, it seems that we can hardly expect an entirely regular and uniform arrangement and separation. And yet, in *biennis* and *biennis sulfurea*, as in *Oe. franciscana sulfurea*, and other forms which I have studied, this seemingly improbable result is accomplished in a great majority of the cells, despite the entire absence of pairing of homologous chromosomes, and the fact that the univalents are still bound together. We find that in each circle, alternate chromosomes become attached by their middles to fibers leading to the same pole, and adjacent chromosomes, therefore, are in contact with fibers leading to opposite poles. As soon as these attachments are formed, the fibers appear to contract. The circles as a result take up a horizontal position across the equatorial region, and since adjacent chromosomes are being pulled toward opposite poles, we get the peculiar and very striking zigzag arrangement shown in figures 16 to 18. Even to the casual observer, the appearance in such a cell is one of regularity. Seven V-shaped chromosomes lie with their apices pointing to one pole, and seven of them point toward the other pole. Each set lies quite neatly in one plane, as seen from the side, and especially in late stages, when metaphase begins to give place to anaphase, a distinct space can be seen between the two sets, unoccupied by chromosomes. To the careful observer, this regularity becomes even more striking when the connections between the chromosomes are discovered, and the fact is noticed that those of one set tend to lie opposite the spaces between those of the other set. And so the chromosomes are for the most part regularly and equally distributed in these plants. As I have pointed out in discussing *Oe. franciscana sulfurea*, it is almost certain that they are so placed within the circles that the zigzag arrangement here described results in the actual separation of the members of every pair of homologous chromosomes.

There is a small percentage of cells in which irregularity in this chromosome arrangement occurs. Such irregularities may be described under three heads (see text-figures 1 to 3, also plate 2, figure 19): (1) In some cases, two adjacent chromosomes in one part of a circle are found to be drawn toward the same pole rather than toward opposite ones, and in another part of the circle, a corresponding pair are ready to pass to the other pole (text-figure 1). The number of chromosomes passing in either direction as a result of this irregularity will in all cases be normal. (2) Another type is where a pair of adjacent chromosomes, as before, are destined for the same pole, and at some other point in the circle, a chromosome is found uncertainly suspended across the equatorial region (text-



TEXT-FIGURES 1 to 3.—Diagrammatic views of the circle of 8 in heterotypic metaphase, with spindles omitted, to show observed types of irregularity in the zigzag arrangement of the chromosomes.

figure 2). This chromosome can apparently go to either pole. If it goes to the one to which the abnormally arranged pair goes, the number of chromosomes passing to that pole will be 8, and only 6 will go to the other. If it goes to the opposite pole, the number going to each pole will be normal, and the result will be the same as in the first type of irregularity. (3) A third sort of abnormal distribution is where two chromosomes in different parts of a circle are found to be suspended between the poles (text-figure 3). If both go to the same pole, 8 will pass to that one and 6 to the other. If they go to different poles, the number passing to each will be normal.

A careful study of some mid-interkinesis stages in *biennis* and *biennis sulfurea*, covering some 500 cells in the case of *biennis*, and over 600 cells in case of *biennis sulfurea*, showed that in about 2 to 5 percent of the cells, an unequal distribution had occurred, so that one nucleus received 8, and the other 6 chromosomes. No cases were observed in which the deviation from the normal number was greater than this, although if such irregularities as I have just mentioned occurred simultaneously in both circles, such might be possible. Since, however, irregularity in the zigzag arrangement of the chromosomes at metaphase does not necessarily result in unequal distribution, it follows that the amount of irregularity must be somewhat higher than the percentage here indicated. As I shall point out later, any irregularity in the zigzag arrangement will probably have an important genetical result, even though the number of chromosomes passing to the poles has not been changed from the normal.

In this connection, some data may be of interest, bearing upon the relative frequency with which the regular zigzag arrangement and deviations from it have been observed. Several slides of *Oe. biennis* were studied intensively with this end in view, every cell found in metaphase being examined carefully, except that the following classes of cells were eliminated from consideration: (1) Polar views, and other cells not affording a side view of the spindle. (2) Incomplete cells, and those in which the chromosome arrangement was obviously disturbed by the knife. (3) Those in which the chromosomes were so badly clumped that nothing could be learned from them. All other cells were critically examined and the results tabulated under a number of headings, as seen in table 1.

TABLE 1

Analysis of a complete survey of the cells in heterotypic metaphase found on several slides of Oe. biennis.

CLASS	DESCRIPTION	NUMBER OF CELLS OBSERVED	PERCENTAGE
1	Both circles in the cell regularly zigzag	47	16.5
2	One circle clear and regularly zigzag, the other wholly or partially absent, or obscure	60	21.1
3	As much of both circles as could be seen, regularly zigzag	113	40.0
4	Mostly regular, with probably some irregularity	15	5.2
5	Obscure, with possible irregularities	20	7.0
6	Quite irregular, not clear	4	1.4
7	One circle regularly zigzag, the other not yet arranged (early metaphase)	5	1.76
8	Regularly zigzag in the main, with clearly observed irregularity of the types described in the text	16	5.6
9	Both circles clear, irregular	4	1.4

TABLE 2

Analysis of class 2 under table 1.

NUMBER OF CHROMOSOMES VISIBLE (ALL VISIBLE CHROMOSOMES REGULARLY ZIGZAG)	NUMBER OF CELLS
Circle of 8, and 5 of circle of 6	3
“ “ “ “ 4 “ “ “ “	8
“ “ “ “ 3 “ “ “ “	5
“ “ “ “ 2 “ “ “ “	1
“ “ “ “ – – –	9
Circle of 6, and 7 of circle of 8	5
“ “ “ “ 6 “ “ “ “	4
“ “ “ “ 5 “ “ “ “	3
“ “ “ “ 4 “ “ “ “	5
“ “ “ “ 3 “ “ “ “	3
“ “ “ “ 2 “ “ “	1
“ “ “ “ – – –	11
Chains of 6, and 2	1
“ “ “ “ 4	1
Total	60

TABLE 3

Analysis of class 3 under table 1.

NUMBER OF CHROMOSOMES IN EACH OF THE TWO CIRCLES CLEARLY OBSERVED TO BE ATTACHED END TO END, AND TO DISPLAY THE REGULAR ZIGZAG ARRANGEMENT	NUMBER OF CELLS
7 and 5	3
7 " 4	1
7 " 3	1
7 " -	6
6 " 5	3
6 " 4	8
6 " 3	5
6 " 2	1
6 " -	17
5 " 5	2
5 " 4	13
5 " 3	10
5 " -	10
4 " 4	10
4 " 3	14
4 " -	6

A further analysis of classes 2 and 3 may be desirable, and this I have presented in tables 2 and 3.

From the tables it will be seen that in a large number of the cells listed under classes 2 and 3, a great majority of the chromosomes were found to be regularly arranged. The proportion of these cells in which irregularities would have occurred is so slight as to be entirely negligible. The fact that classes 1 to 3 include 77.6 percent of the cells examined, and that 37.6 percent showed at least one regularly arranged circle in its entirety, proves rather convincingly that the zigzag arrangement in metaphase is a constant feature in these plants.

The circles do not break up until the chromosomes are pulled apart in anaphase. This phase lasts but a short time. The chromosomes are generally in the form of short V's as they pass to the poles (plate 2, figure 20), though in cases where there is an irregular arrangement in metaphase, some may pass to the poles as rods, the spindle fibers being attached near their ends. In most cases, no sign of a longitudinal split can be detected. In a few cells, however, its presence has been clearly indicated (figures 21 and 22), and it is probable that the chromosomes become split at this stage or earlier, but that in most cases the halves do not separate sufficiently at this time to make the fact evident.

Interkinesis and subsequent stages

The chromosomes, having arrived at the poles, are gathered together into a compact group and a nuclear membrane appears, enclosing them. When seven chromosomes are present, one tends to assume a central position, with the other six occupying the periphery (figure 24). The nucleus then grows rapidly in size until the maximum is reached. In the few cells in which the split nature of the chromosomes becomes evident in anaphase, they continue in early telophase to exhibit this characteristic (figure 23). In most cells, however, such a split has not as yet become visible. In these, the chromosomes lose at this time the V shape that characterized them during anaphase, and assume instead a more or less squarish appearance. They are very clearly defined, and at first have no connection with one another. About the time of the formation of the nuclear membrane, however, we notice that delicate little threads tend to pass out from the chromosomes, especially at the corners, and to fuse with one another, so that the chromosomes in the little cluster become slightly attached (figures 23 and 24). The formation of these threads is at once followed by an expansion of the chromosomes, which generally takes the form of an elongation and enlargement of the threads just formed (figures 25 and 26). Since these have developed at the corners as a rule, each chromosome soon begins to look like an irregular kind of cross. The chromosomes do not remain long attached, for the nucleus grows faster than they expand, and each chromosome tends to lie close to the nuclear membrane. Hence, the delicate threads by which they were joined soon break. If the expansion of the chromosomes were to keep pace with the growth of the nucleus, we might conceivably have developed a reticulum, but as it is, no reticulum is formed. At first the chromosomes are ragged, irregular structures, with the black-staining chromatin material unevenly scattered. When the nucleus has finally reached its maximum size, however, and the chromosomes their maximum degree of differentiation, they have a more definite shape. Each chromosome looks like a Maltese cross. The ends of the arms are swollen, and one or two additional swellings may be present in each arm. Usually the center of the chromosome looks solid. But in some, it can be clearly seen that there is a split running through the center. The chromosome is really a double affair, made up of two elongated parts. These have separated widely from each other at the ends, but they are still more or less intimately associated toward the center. Two of the arms, therefore, belong to one granddaughter chromosome, and two to the other.

There is some evidence at this stage to indicate that all of the chromosomes are not constructed in exactly the same manner. In some, the two halves are attached at the mid-region, and the two ends of each half are alike in size and shape. In others, the halves are attached at a region close to one end, so that one end of each half is about twice as long as the other. The difference between the two conditions is not very striking, so that unless one can study all seven chromosomes at once, it is not possible to say definitely whether it is at all significant. Unfortunately, on account of the fact that in every nucleus some of the crosses are seen edge on, it is not ordinarily possible to study a whole complex of chromosomes together, and so it cannot be stated definitely that visible structural differences in the chromosomes exist at this stage.

During early interkinesis, one or more nucleoli develop in each nucleus. These are small, round, and weakly staining, and invariably appear in contact with the chromosomes. They continue until the nucleus begins to shrink, then disappear. From their small size and empty appearance, it would seem that they are perhaps nearly if not quite functionless in these nuclei.

Judging from the frequency with which the stage is seen, mid-interkinesis lasts for a considerable period. In time, however, the chromosomes begin slowly to shrink, the X's becoming smaller and smaller; and finally the nucleus as a whole contracts rather quickly, crowding the chromosomes into a cluster at the center (figure 27). Then the nuclear membrane disappears, and the spindle fibers which have meanwhile formed in the cytoplasm penetrate and become attached to the chromosomes. At first the spindle is multipolar, but soon becomes bipolar, and the chromosomes are carried to the equatorial region and lined up in a regular fashion (figures 28 and 29). By this time, they have shrunken until they are very small indeed. Furthermore, the separation between the granddaughter chromosomes is often becoming very clearly marked. Each half resembles a small dumb-bell, since it is somewhat swollen at the ends, with a constriction in the middle. The two halves pair together perfectly during metaphase. The two homoeotypic spindles formed in the same cell may be in one plane or in different planes, often at right angles to each other.

The granddaughter chromosomes, as they pass to the poles, are regularly V-shaped, although sometimes they are in the form of rods (figure 30). At the poles, they are gathered into small clusters around which nuclear membranes are soon formed. As was the case in the heterotypic telophase, when seven chromosomes are present, one tends to occupy the center, with the other six surrounding it (figure 31).

As the four nuclei increase in size, the chromosomes undergo a process of elongation, until they reach a considerable length (figures 32 and 33). Then they begin to put forth delicate threads which anastomose, and form a loose reticulum. The chromatin material begins to move out into these threads, and little by little, a clear and prominent network is developed (figures 34 and 35). A large part of the chromatin material of each chromosome remains in one mass, but it becomes so irregular, and the material passing out into the threads forms other accumulations of such a nature that it soon becomes difficult, if not impossible, to distinguish the individual chromosomes or to tell what is the chromosome number. The return of the chromosomes to the reticulate condition in these cells does not conform to the processes as described in other plants by SHARP (1920), DIGBY (1910, 1919) and others. We have here a very clear formation of thread-like, anastomosing outgrowths from the chromosomes, which form the basis for the reticulum. This is quite different from the processes of internal expansion involving vacuolization, or splitting and attenuation of the chromosomes, such as described by these authors.

Nucleoli appear during this process, in contact with the chromosomes. At first they are small and weakly staining, but in time they attain to a fair size, and take the haematoxylin stain very strongly. They appear to arise *de novo*, from the material composing the chromosomes.

No cell walls are developed in the pollen mother cells of these plants until the granddaughter nuclei have been formed, and have passed more or less fully into the resting condition. The nuclei occupy positions near the periphery of the cell, and are about equidistant one from the other. The first sign of cell division is an invagination in the surface of the protoplast along lines midway between the nuclei. It is not until these invaginations have become quite large that cleavage planes appear. When they do appear, they apparently begin at the surface, and cut rapidly through to the center. Thus, the four pollen cells are formed simultaneously by a process of invagination followed by cleavage of the protoplast.

DISCUSSION

Cytological

Two facts of primary cytological interest have been emphasized in the cases of *Oe. franciscana* and *Oe. franciscana sulfurea* (CLELAND 1922, 1924). These are (a) the presence of telosynapsis, rather than parasynapsis, and (b) the regularity that characterizes the whole process of meiosis in the pollen mother cells.

(a) The cytologists who have worked on meiosis in the Oenotheras, with the exception of BOEDIJN (1924), have described the process as telosynaptic. The plants under discussion in this paper have proved no exception to the rule. Throughout all stages of the meiotic prophase in the anthers there is a lack of the sort of evidence that is necessary to prove the presence of parasynapsis. Neither pre-synizetic nor post-synizetic stages are characterized by parallelisms or splitting to any noticeable extent. This is particularly true of the open spireme stage. Added to these facts we have the evidence presented by second contraction and "diakinesis" stages. The radiating loops which appear in very early second contraction can be observed in every stage until the unfolding of the central mass, and the beginning of "diakinesis." No longitudinal splitting occurs in these loops as far as can be seen. They apparently become transformed directly into chromosomes which are strung end to end, and constitute parts of the spiremes which finally emerge. Thus, we can establish a clear relationship between the threads of the open spireme and those later, haploid spiremes which characterize the stage of "diakinesis." The very persistence of an end-to-end condition in "diakinesis" is in itself a strong piece of evidence in favor of telosynapsis. While it would be too much to say that the development of an arrangement such as is found here would not be possible under conditions of parasynapsis, it is extremely improbable that such a striking end-to-end arrangement of all 14 chromosomes would result, were the homologous chromosomes placed side by side in the open spireme. All of the evidence, therefore, seems to favor a telosynaptic interpretation in these plants.

(b) One of the most interesting things about meiosis in the Oenotheras is the regularity with which its processes are carried out, and this in spite of the fact that in many important respects they are quite different from the corresponding processes in most plants. The stages which are most at variance with what we find in other forms are diakinesis and the first maturation metaphase.

By diakinesis we usually mean a period during which the homologous chromosomes are closely paired, and the pairs widely separated from one another. This condition is altogether wanting in *biennis* and *biennis sulfurea*, there being in these forms an entire absence of pairing. In spite of this, however, we find no lack of uniformity. In all cells, with apparently few or no exceptions, the unpaired chromosomes are arranged end to end into closed circles, one of 6 and the other of 8 chromosomes, and the circles are as a rule linked together. The constant presence of such configurations as these is as striking as it is unusual. It probably means that in earlier stages in prophase the network has a definite structure, such that when it condenses to form the spiremes, the individual chromosomes come out in the same way each time.

Nor is regularity of behavior confined, in all probability, to the arrangement of the chromosomes into two circles, each of a definite number. As I have pointed out in the case of *Oe. franciscana sulfurea*, there is little doubt that the chromosomes occupy definitely fixed positions within the circles also, for, with adjacent chromosomes going to opposite poles, the number of cells in which non-disjunction would occur if the chromosomes were placed in the circle by chance would be impossibly high.

In metaphase, the regularity of behavior is fully as striking as it is in the immediately preceding stage, perhaps more so, in view of its peculiarity. As in *Oe. franciscana sulfurea*, so here, we find that adjacent chromosomes are caught by fibers leading to opposite poles, and thus are separated in anaphase. I have already described the process. It remains only to emphasize the fact that the chromosomes are not brought to the equatorial region in a haphazard manner, and distributed apparently by chance, but the whole process from beginning to end is orderly and strictly according to rule, the occasional irregularities only serving to lay stress upon the conspicuous uniformity in behavior.

The orderly distribution of the chromosomes comprising the circles, to the poles, brings up the interesting question regarding the mechanism by which this is accomplished. We have a decidedly different problem here from that connected with the separation of half chromosomes in somatic mitoses, or of closely paired homologous chromosomes in meiosis. In neither of these cases does there appear to be any element of selection. In somatic mitoses, as far as we can see, either half of a split chromosome may pass to a given pole; and as for meiosis, aside from any cytological evidence supporting the theory that a given chromosome of a homologous pair may go to either pole, the laws of independent assortment of genes require that such be the case if the heterotypic division is to satisfy all requirements as the mechanism which is responsible for these laws. But in the case of the circles in Oenothera, the element of selection enters in. As a rule, every second chromosome is caught up on spindle fibers leading to a given pole, and carried to that pole. Chance apparently does not determine the direction in which a particular chromosome shall go, nor does proximity of the chromosomes to one pole or the other, as they lie in the newly formed bipolar spindle, have anything to do with it. There is some force of attraction between a given pole and certain chromosomes, or there is some other mechanism, as yet unexplained, which results in the definite selection of a certain set to be taken to one pole, and another set to be carried to the other. Any theory, it seems to me, that attempts to explain the separation of chromosomes and their passage to the poles, must take into account this peculiar selection among the unpaired chromosomes of the Oenotheras.

Genetical

The probable reason for the failure of chromosomes to pair with their homologues in "diakinesis"

In *Oe. biennis* and *Oe. biennis sulfurea* not a single chromosome succeeds in pairing with its homologue. This is far from being a universal condition in the evening primroses, however; in fact, it is true in only three of the eleven species or varieties which I have thus far studied (CLELAND 1923, 1925). All conditions are found in the various species from complete absence of pairing, as here, to the presence of seven pairs, as in *Oe. blandina* and *Oe. deserens*. A given chromosome pair, then, does not necessarily behave in the same way in every species. In some, the individual chromosomes can pair, in others, they cannot. Why do chromosomes fail to pair in some species, and why do they often behave differently in the different species?

One might conceivably argue that the failure is due to the fact that the bonds of union between the telosynaptically arranged chromosomes are stronger than the tendency to pair, which tendency might even be considered as strong in the evening primroses as in other plants, but not strong enough to overcome the physical strength of the attaching threads. This seems to me to be untenable, however, for it cannot explain the regularity of chromosome arrangement in the various species which possess not only a circle, but a definite number of pairs as well; and it fails particularly in view of the fact that while in one species a certain set of homologues always break away from the spireme and pair up, in another species they are unable to do so.

At present I am holding to another theory, which seems to fit all the facts at my diposal. It is that the failure of the homologous chromosomes to pair is due to lack of affinity, and that this incompatibility between the homologues is the result of divergence in their constitutions. In other words, chromosomes which pair in diakinesis in Oenothera are relatively homozygous. Those which fail to pair are relatively heterozygous. Some of the reasons for adopting this theory are: (1) The only species that I have studied which have perfect pairing (*Oe. deserens* and *Oe. blandina*) are entirely homozygous and have but one type of gamete. (2) *Oe. franciscana,* with its freedom from mutants, its uniform gametes, and its high percentage of seed and gamete fertility, has a large number of paired chromosomes, namely, 5 pairs, and a circle of 4 which in metaphase usually breaks into two pairs. (3) Some of the species that I have examined are known to be permanently heterozygous. All these have displayed circles, with partial to complete absence of paired chromosomes. (4) Using this theory as a basis, it is possible to explain the origin of certain mutants from parent forms. A discussion of this I shall reserve until a later paper. (5) The failure of homologous chromosomes to pair in diakinesis is characteristic of a good many hybrids, where there is every reason to believe that the corresponding chromosomes descended from the two parents are decidedly diverse in nature. The similarity between the cytological behavior in many Oenotheras and in such hybrids suggests that the lack of pairing in both is due to similar causes.

There is not room in this paper for a discussion of the possible origin of chromosome incompatibility in the Oenotheras. It may merely be stated at this time that it is perhaps not necessary to assume that it arose as the result of hybridization, but it may have come about in other ways, as for instance through the gradual accumulation of gene mutations within the chromosomes, a process aided probably by the appearance of balanced lethal factors. It may well be asked in this connection why a similar failure to pair has not commonly arisen in plants. We must assume either that gene mutations have for some reason taken place much more frequently in the evening primroses than in other organisms, or else that it requires fewer changes in an Oenothera chromosome to make it incompatible with its mate than is the case with the chromosomes in other plants. It may also be that it is more difficult for chromosomes to pair when they are arranged end to end, than it is when the homologues are already placed side by side in the spireme.

The genetic composition of *Oe. biennis* in relation to its chromosomes

Oe. biennis is one of the permanently heterozygous species of evening primrose. It appears to have two distinct types of gamete, but on account of the presence of lethal factors, the complexes carried by these cannot exist in the pure state and hence only the combination of the two survives. The result is a permanent heterozygote, which breeds true in the main. There seems to be a difference of opinion in regard to the nature of the lethal factors involved. RENNER (1917) found that both complexes can exist in the egg, but only one of them in the sperm. One of the complexes would thus possess a sperm or pollen lethal, the other a zygote lethal. De Vries's (1913) experiments, on the other hand, would indicate that there is but one kind of successful sperm, and one kind of egg, and that the kinds are different. One complex, then, would have a sperm or pollen lethal, the other an egg lethal. In either case, there is but one kind of successful pollen grain, or at any rate, the pollen gives rise to sperm of only one sort.

The diploid number of chromosomes in *Oe. biennis* is 14. Theoretically, species with this number ought to be able to produce as many as 2^7 different kinds of gametes, depending upon the number of pairs of homologues that happen to be heterozygous in a given individual. As few as two kinds of gamete could be produced under two conditions. First, a plant might have but one heterozygous pair, the other six pairs being homozygous. Second, all of the heterozygous chromosomes might be linked together and act as a unit in heredity. The difficulty with the first possibility is that the possession of only two kinds of gamete is characteristic, not of a small proportion of individuals, but, so far as we know, of all of them. It is unlikely that all plants of *Oe. biennis* are heterozygous in one and the same chromosome, and homozygous in the case of the other six. The other possibility seems *a priori* fully as unlikely. Nevertheless, the cytological evidence undoubtedly favors the second explanation. It is probably a fact that all of the chromosomes in *biennis* and *biennis sulfurea* are linked in such a way that they act as a unit in heredity.

I have already emphasized the probability that the chromosomes are arranged within the circles in a definite order, so that homologues are as a rule separated to opposite poles. This order probably applies not merely to the position of the pairs of homologues, but to that of individual chromosomes as well, so that each chromosome occupies a given position and does not ordinarily change position with any other chromosome, even with its homologue. Supposing this to be the situation, and recalling the fact that unpaired chromosomes are probably more or less heterozygous, let us call the four pairs which comprise the circle of eight AA′, BB′, CC′, and DD′; and let us assume that they are arranged within the circle in the following order—AA′BB′CC′DD′. In like manner, the circle of six can be lettered EE′FF′GG′. We know that, as a rule, adjacent chromosomes are separated and pass to opposite poles. In the circle of eight, therefore, ABCD will pass to one pole, and A′B′C′D′ to the other. Since the arrangement of chromosomes into circles seems to be constant, not only in all pollen mother cells of a given individual, but apparently in the various individuals of the species, we may conclude that if in one cell chromosomes ABCD pass to one pole, and A′B′C′D′ to the other, they will be distributed after the same fashion in all normally behaving pollen mother cells throughout the species. This being the case, ABCD are as much linked in all pollen mother cells as though they were parts of a single chromosome, and the same applies to A′B′C′D′, EFG and E′F′G′. It is as though we had but two sets of homologues, then, instead of seven.

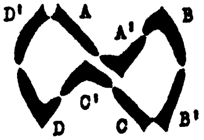
But if these two sets act independently, as do the various sets of homologous chromosomes in other plants, four kinds of gamete will be possible, ABCDEFG, ABCDE′F′G′, A′B′C′D′EFG and A′B′C′D′E′F′ G′. This does not satisfy the genetical situation, for it has been shown that there are apparently but two types of microspore formed. In all of the other species that I have examined, in which there are unpaired chromosomes, these are grouped into but one circle, and the formation of only two kinds of gamete is easily explained. But *biennis* and *biennis sulfurea*, in having two circles, have proved exceptions to this rule, and an exact cytological explanation of the presence of but two types of gamete is therefore not so obvious. In order to bring chromosome behavior into line with genetical discoveries it is necessary to assume in the case of these species that a given chromosome complex resulting from one circle is under the necessity, ordinarily, of going to a given pole, along with a certain complex from the other circle. In other words, it is not a mere matter of chance whether EFG go to the same pole as ABCD or to the opposite one. When we stop to consider, this is what we would naturally expect in the circumstances. Chromosomes ABCD are apparently selected from the circle of eight and carried to one pole because there is some sort of attraction between them and that pole, and the other chromosomes are as much attracted by the other pole. The same applies to the circle of six. Each circle, then, has one set of chromosomes with affinities for each pole. Thus, we may say, for instance, that the complexes ABCD from one circle and EFG from the other have like affinities, and consequently pass to the same pole. But if this is true in one cell, it is no doubt true in all, for the complexes will surely possess the same characteristics in all normal pollen mother cells. The complexes from the two circles do not act independently, therefore, but the same chromosomes go to the same pole in all pollen mother cells, even though they be separated morphologically into two groups. Two complexes are formed as a result, which we shall letter ABCDEFG and A′B′C′D′E′F′G′. Only one of these complexes is able to survive in the sperm, however, owing to the presence of a lethal factor, so that but one kind of functional male gamete is formed,—and that not because there is present in all cells but one heterozygous chromosome pair, but because all of the chromosomes are in effect grouped into one great linkage system, such that the same chromosomes go, as a rule, to the same pole in all cells, and form complexes corresponding to the two complexes which RENNER has called *rubens* and *albicans*. It can be said, then, that the genetical composition of *Oe. biennis* may apparently be explained on the basis of the behavior of the chromosomes during meiosis in the pollen mother cells, since the peculiar type of linkage which goes along with the presence of but two sets of gametes seems to be due to the way in which the chromosomes are linked, and then separated, in the heterotypic mitosis.

The genetical significance of irregularities in the zigzag arrangement of chromosomes

As I have already suggested, in a certain percentage of cases there is a departure from the regular zigzag arrangement of chromosomes in the heterotypic metaphase. I have already described three types of irregularity (text-figures 1 to 3) and brought out the fact that, as far as numerical distribution to the poles is concerned, these may in some cases result in abnormalities, although this is not necessarily the outcome. But even when irregularities of the various sorts do not cause a deviation from the number of chromosomes normally going to the poles, they nevertheless result in all cases in a distribution that is in other respects abnormal, as we shall see.

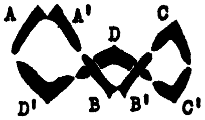
Wherever the number carried to the poles is 8 and 6, instead of the normal 7 and 7, the grains containing six chromosomes will probably prove functionless, or at least will give rise to functionless embryos. No 13-chromosome Oenotheras have ever been found, so far as I am aware. The 8-chromosome pollen grains, however, if they happen to possess the successful *rubens* complex, rather than the unsuccessful *albicans* one, may conceivably succeed in giving rise to individuals bearing 15 chromosomes, providing only that the extra chromosome which it has received is not the one possessing a lethal. If a successful 15-chromosome individual should arise, one of its sets of homologues would possess three members instead of two, and of these, two would belong to the *albicans* complex. Any recessive factors contained in the duplicated chromosome would probably express themselves under such circumstances, and a taxonomic character somewhat different from the normal might be displayed by the plant.

We have now to discuss the result of irregularities in *Oe. biennis* in cases where the number of chromosomes distributed to the poles is normal. I shall first suggest something of the variety of types of chromosome complex that can arise, and then indicate the probable genetical result of the formation of such complexes. I have taken for illustration the first of the three types of irregularity described in this paper. Several figures are presented herewith for purposes of illustration (text-figures 4 to 7). In all of the figures, the order in which the chromosomes are arranged within the circles has been kept the same, since, as I have stated before, I conceive this order to be constant.



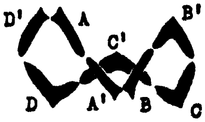
$$\frac{A\ B\ C'D'}{A'B'C\ D} + \frac{E\ F\ G}{E'F'G'} = \frac{A\ B\ C'D'E\ F\ G}{A'B'C\ D\ E'F'G'}$$

4



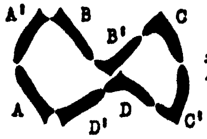
$$\frac{A\ A'C\ D}{B\ B'C'D'} + \frac{E\ F\ G}{E'F'G'} = \frac{A\ A'C\ D\ E\ F\ G}{B\ B'C'D'E'F'G'}$$

5



$$\frac{A\ B'C'D'}{A'B\ C\ D} + \frac{E\ F\ G}{E'F'G'} = \frac{A\ B'C'D'E\ F\ G}{A'B\ C\ D\ E'F'G'}$$

6



$$\frac{A'B\ C\ D}{A\ B'C'D'} + \frac{E\ F\ G}{E'F'G'} = \frac{A'B\ C\ D\ E\ F\ G}{A\ B'C'D'E'F'G'}$$

7

TEXT-FIGURES 4 to 7.—Diagrams showing 4 of the 20 possible pairs of complexes that may arise when an irregularity of the type seen in text-figure 1 occurs in a circle of 8 chromosomes, while the circle of 6 is regularly distributed. Complexes resulting from the circle of 8 are shown immediately to the right of the diagrams, and the total complexes derived from both circles are given at the extreme right. Chromosomes above the horizontal lines go to the upper pole, those below the lines to the lower one.

In determining the total number of sets of complexes that can be formed as the result of the type of irregularity chosen, four factors which tend to increase the number must be taken into consideration. First, from the standpoint of their position in relation to the individual chromosomes, it is theoretically possible for the irregularities to occur at any point in the circle. Thus, in text-figures 5 and 6, the irregularities are identical in kind, but they have occurred at different points, and the resulting complexes are therefore different. Second, the two abnormally arranged pairs may occupy different positions with reference to each other in the circles (compare text-figures 4 and 5). Third, since there are two circles in each cell, the position that the irregular complexes resulting from a circle occupy with reference to the poles must be taken into account. Thus, it will be noted that the complexes resulting from the circle of eight are alike in text-figures 6 and 7, but since they are carried to different poles in the two cases, they become associated with different chromosomes from the circle of six, and the total complexes are therefore different. Fourth, irregularities may occur in both circles in a cell, which greatly increases the number of complexes that are possible.

If one, taking these factors into consideration, will work out all of the possibilities, he will find that in the case of a circle of eight chromosomes the type of abnormality which we are discussing may result in as many as 20 different sets of complexes; and that in a circle of six, such an irregularity may give rise to any one of 12 different sets. In half of these sets (see text-figure 5), it will be found that one chromosome is entirely missing in each complex, whereas both members of another pair are present. Since none of these chromosome sets is complete, the probability is strong that many, if not all, will render the gametes bearing them incapable of functioning. For this reason I shall eliminate these cases from the discussion and deal only with those in which full complements are formed.

Cells in which the circle of 8 alone displays irregularity will give rise to any one of ten different sets of chromosome complexes in which both complexes will receive one member of every chromosome pair. Those in which such irregularity is confined to the circle of 6 can result similarly in any one of six different ways. Altogether, then, cells with irregularity in only one circle may give rise to pollen grains possessing any one of 16 different sets of complete chromosome complexes. But irregularities may occur in both circles at the same time. The total number of sets that are possible in such an event is the product of the numbers possible when the irregularities are confined to one circle, or $10 \times 6 = 60$ sets. The total number of sets of complexes possessing a full complement of chromosomes, and resulting from the type of irregularity that I have chosen, is therefore 76. One complex out of each set of two will probably fail to survive, since it will include the chromosome containing the sperm lethal. 76 types of sperm are therefore theoretically possible as the result of irregularities in one or both circles. These are all abnormal types of gamete, deviating in some way from the normal ABCDEFG type. Some of the complexes resulting from such irregularities will differ from the normal in respect to only one chromosome (text-figure 7). Others will differ in respect to two or three chromosomes (text-figures 4 and 6). Greater deviation is not possible where only seven pairs are involved, and where each complex has a full complement of chromosomes.

We may now discuss the possible genetical significance of such irregularities in chromosome behavior. Suppose that we let the chromosome complex ABCDEFG represent the *rubens* gamete, and A′B′C′D′E′F′G′ the *albicans*. Suppose further that an irregularity such as is figured in text-figure 7 has occurred in a certain pollen mother cell, and that the pollen grains which result are among those used in a cross with another species. If A′ does not carry the *albicans* sperm lethal, and the sperm from these grains succeed in fertilizing eggs, the resulting plants will probably exhibit crossing over with respect to certain characters, for the factors contained in A′ will show up in these individuals instead of those contained in the normally present A. As we have seen, ABCDEFG are normally carried to the same pole in the heterotypic anaphase. Genes contained in these chromosomes are therefore linked in inheritance. Each chromosome is to the whole linkage system what a chromomere is to a single chromosome. In the case shown in text-figure 7 there has been an exchange of the members of one chromosome pair, comparable to the transfer of chromomeres or sections of chromomeres to corresponding positions in opposing chromosomes. The latter phenomenon is called crossing over. The former we will term "chromosome exchange." The result, in crosses, of crossing over and of chromosome exchange will be much alike, except that in the case of the interchromosomal type, more characters may be involved than would be the case if only a section of a certain chromosome pair were affected.

The result of interchromosomal crossing over in cases where the resulting pollen grains are used for selfing purposes, or for crossing between two plants of the same species, is worth noting. Using text-figure 7 again for illustration, when complex A′BCDEFG is used for purposes of self-pollination, an individual resulting from its union with the normal A′B′C′D′E′F′G′ complex will have two A′ chromosomes, but no A chromosome. Dominant characters, dependent upon genes in A will be lacking in this individual. Furthermore, the A′ chromosomes will undoubtedly be identical, both having been derived from the same plant. Any recessive characters that have lain hidden in A′ because of the fact that, in common with the rest of the complex, it normally cannot exist in the homozygous state, may now show out, and the probability is that in one or more characters this individual may deviate somewhat from the normal run of *biennis* plants. All of this will be true, of course, only provided A′ does not possess a lethal factor. If a pollen grain containing this complex, instead of being used in selfing, is transferred to another plant, which, however, has been derived fairly recently from the same source, the two A′ chromosomes in the zygote will still undoubtedly be almost, if not entirely, identical and the same result may be expected. On the other hand, if the *biennis* plants that are used as parents are of widely different origins, the probability is that the two A′ chromosomes will be decidedly unlike, owing to the fact that gene mutations have been occurring in them, and have been accumulating, thanks to the enforced condition of heterozygosity; and these mutations will almost certainly have been different in the two cases. The likelihood of recessive characters being found in double dose, and showing out, will therefore be reduced.

From the cytological standpoint, we may expect to find that in any individuals where the two A′ chromosomes are almost or quite identical, these will leave the circle of 8, and pair up; but in individuals in which they are decidedly different they may fail to pair, and the circle of 8 will be formed as in normal individuals.

In cases where the number of chromosomes exchanged between the complexes is greater than one, the effect will be greater. A larger block of characters will show the effect of crossing over in crosses with other species, and the number of recessive characters that may show out as the result of self-pollination will be increased. In fact, it is conceivable that the number might be so large as to greatly modify the appearance of the resulting individual, and cause it to be regarded as a mutant. I believe that certain mutants in some species of Oenothera have probably arisen in just this way. Here, again, we should expect pairing of all homologous chromosomes which are identical in composition, so that an individual arising through self-pollination from the union of the complex AB′C′D′EFG (text-figure 6) with the normal A′B′C′D′E′F′G′ complex would be expected to have three paired sets of homologues, B′B′, C′C′ and D′D′.

The chance that a given irregularity will occur in *biennis* is very small indeed. One of the three types of irregular arrangement in metaphase that I have mentioned can result only in the normal number of chromosomes going to the poles (text-figure 1). Two of the types, however, will probably result, in about 50 percent of the cases, in an unequal distribution. An examination of interkinesis stages shows that in about 2 to 5 percent of all the cells an unequal distribution has occurred. If the three types of irregularity that I have described (text-figures 1 to 3) are the only three types, and if they occur with about the same frequency, those resulting in unequal distribution will be found in approximately one-third of the total number of cells in which irregularities have taken place. We may then estimate the proportion of cells in which the irregularities have failed to alter the number of chromosomes distributed as about 4 to 10 percent of the total number of pollen mother cells. In order to render the figuring simple, let us choose 8 percent as an approximate average of these. We have seen that there are 20 such irregularities possible in the circle of 8, and 12 in the circle of 6. The ratio of the possible irregularities in the two circles is 5 : 3. If the number of cells in which they occur totals about 8 percent of the whole, there will be about 5 chances in 100 that the circle of 8 will be irregularly arranged without altering the chromosome number in resultant cells, and about 3 chances in 100 that such irregularity will occur in the circle of 6. Each of the 20 possible irregularities in the circle of 8 will have one chance in 400 of appearing, and each of the 12 possibilities in the circle of 6 will also occur on the average in one cell in 400. The chance that irregularities will occur in both circles in the same cell is much smaller. There is only one chance in 660 of such an occurrence, and the probability of any particular combination of the possible irregularities in the two circles taking place is about one in 160,000; or in other words, it will appear on the average once in about 160,000 cells.

I do not claim, of course, that these figures are even approximately accurate, for I do not know whether there may not be more kinds of irregularity than the three that I have described, and I do not know the relative frequency with which these types occur. Certain conditions may favor the appearance of certain irregular chromosome complexes more often than others. Nevertheless it can be readily seen that the occurrence of any given irregularity is likely to be very rare indeed. While this is true of each individual irregularity, however, there will no doubt be a considerable proportion of cells that will experience some one of the numerous sorts of deviation capable of producing functional gametes. Approximately half of the sperm of normal chromosome number resulting from irregularities will have full complements of chromosomes and should prove functional. These will probably amount, therefore, to 3 or 4 percent of the total number of sperm, and consequently a number of plants in any given strain should show in some degree variations from the normal and uniform appearance.

Summing up the effect of irregular arrangements in metaphase, therefore, we may say that they cause the exchange of one or more chromosomes from one complex to the other, a phenomenon that I have called "interchromosomal crossing over." Resultant reproductive cells which lack sperum lethals and have a complete set of chromosomes will probably prove functional, while all others will be incapable of functioning. In crosses with other species, the functioning of such gametes will result in the appearance of crossovers. In the case of self-pollination, the individuals resulting from the union of the modified complexes with normal gametes will vary from the normal in a degree depending upon the number of chromosomes which have crossed from one complex to the other. In the case of maximum transference, the resulting individual may perhaps be so different that it will be regarded as a mutant. The chances of such mutants appearing, however, are very slim. I have made no effort in this paper to calculate what might happen if a modified male gamete were to meet an egg likewise altered through the irregular distribution of the chromosomes. Not having studied meiosis in the embryo sac, I do not know, of course, that the same mechanism is found there.

The presence of chromosome exchange does not exclude in any way the possibility of the presence of crossing over of the usual type. In some cells in late second contraction, I have noticed that the appressed sides in the case of one or more of the peripheral loops have apparently become twisted about each other in a way that might conceivably result in the exchange of chromatin particles. With both types of crossing over cytologically possible, it would not be surprising to find cases of crossing over in *Oe. biennis* and *Oe. biennis sulfurea.*

The relation of *Oe. biennis sulfurea* to *Oe. biennis*

The arrangement of chromosomes in "diakinesis," and their behavior in metaphase are identical in the two forms. It can be safely assumed, therefore, that the *sulfurea* factor has arisen as the result of a gene mutation, rather than through chromosomal aberration. The foregoing discussion of *biennis* will therefore hold in all points for *biennis sulfurea.*

SUMMARY

Descriptive

1. In early prophase stages of both *Oe. biennis* and *Oe. biennis sulfurea,* the delicate network of the resting nucleus is transformed into a relatively thick-threaded, large-meshed reticulum, the so-called "open spireme." The process apparently involves the contraction of some threads, and their absorption into others; and the stresses and strains thus set up may be the cause of the synizetic contraction; or perhaps they do not in themselves cause shrinkage, but rather make it easy for the fixing fluid to

do so, in which case the contraction is to be regarded in the nature of an artifact.

2. The nucleoli, of which one is usually much larger than the others, are in intimate contact with the thread system, and probably contribute largely to the chromatin content of the network during this process. An endonucleolus becomes visible in late synizesis, and is frequently seen to have an organic connection with the reticulum.

3. With the approach of second contraction, a condensation of chromatin material occurs near the center of the nucleus, and peripheral loops become evident. These loops may be traced throughout the contraction stage, and when the knot unfolds, they are found to be parts of the spiremes which emerge.

4. In none of these stages do we find evidence of extended approximation of threads, or of splitting. The spireme is undoubtedly univalent in character, rather than bivalent, and the situation calls for a telosynaptic interpretation.

5. In late prophase, after second contraction, we find two closed circles, one composed of eight, and the other of six univalent chromosomes attached end to end. The two circles are linked, but may separate later. This arrangement is constant, and apparently characteristic of both of the species in question. Occasionally the circles are found to have broken open, forming chains of six or eight, rather than closed circles. In no cases do homologous chromosomes pair at this stage, as they do in diakinesis in most plants.

6. The circles usually remain intact throughout the heterotypic metaphase. After the formation of the bipolar spindle, alternate chromosomes become attached to spindle fibers leading to the same pole, adjacent chromosomes to fibers leading to opposite poles. In mid-metaphase, the circles lie horizontally across the equatorial region, and present a regular zigzag appearance, due to the fact that adjacent chromosomes are being pulled to opposite poles.

7. Irregularities in the zigzag arrangement are occasionally found, and have been described in the text.

8. Anaphases present for the most part a regular appearance. Seven chromosomes pass to each pole, except that in about 2 to 5 percent of the cases, eight go to one and six to the other, due to irregularities in the zigzag arrangement.

9. During interkinesis, the daughter nuclei increase in bulk, and the chromosomes become transformed into structures resembling Maltese crosses. Two of the arms of each cross belong to one granddaughter

chromosome, and two to the other, and the split between the two halves can be clearly seen in many cases. Chromosome individuality is clearly preserved throughout interkinesis.

10. The homoeotypic divisions occur simultaneously, the spindles lying in the same or in different planes. It is not until after the granddaughter nuclei have attained to the resting condition, and most traces of chromosome individuality have been lost, that walls are formed. The return of the chromosomes to the reticulate condition of the resting stage involves neither a process of splitting nor of vacuolization, but threads are put forth from neighboring chromosomes which amalgamate, and in this way a network is formed.

Discussion

11. While the behavior of the chromosomes during "diakinesis" and the heterotypic metaphase is quite out of keeping with what is found in most plants, it is in the main very regular and there is nothing hit-or-miss about it.

12. The chromosomes are undoubtedly arranged in a definite sequence within the circles, otherwise the amount of non-disjunction that would occur with adjacent chromosomes going to opposite poles, would be impossibly high.

13. Every chromosome seems to have some sort of affinity for a particular pole, otherwise the picking out of alternate chromosomes to be sent to the same pole is inexplicable. Each chromosome must go to one pole rather than the other, and as this applies to every chromosome in the cell, it naturally follows that in every cell the same chromosomes will go together to the poles. The effect will be the same as though the chromosomes were actually bound together structurally in such a way as to form one pair of large compound chromosomes.

14. The probable reason why homologous chromosomes fail to pair in "diakinesis" in the Oenotheras is because they are incompatible, and this incompatibility is presumably due to divergence in their constitutions. Relatively heterozygous chromosomes fail to pair, relatively homozygous ones are capable of pairing. In *biennis* and *biennis sulfurea*, therefore, all the chromosomes are probably relatively heterozygous.

15. *Oe. biennis* is a permanently heterozygous species, possessing but two recognizable types of gamete, which RENNER has called *rubens* and *albicans. Albicans* fails to survive in the sperm condition, so that all functional male gametes possess the *rubens* complex. In order that only two types of gamete shall be formed in a plant, all heterozygous genes

must be linked in one chromosome; or if they are in more than one, the chromosomes possessing them must be linked. In *biennis* and *biennis sulfurea* the fact that none of the homologous chromosomes are capable of pairing makes it seem probable that the heterozygous genes are distributed throughout all of them.

16. All the chromosomes, despite their being in two circles, will act in heredity as though they were linked, in view of the fact that alternate chromosomes are as a rule sent to the same pole, provided that they are arranged in a definite order within the circles, and that the reason for their being distributed as they are is because of an affinity or attraction of some sort between each chromosome and a given pole. Both of these suppositions appear to be justified, so that the conclusion is admissable as a working hypothesis. According to this theory, if pairs of homologous chromosomes are represented by AA′, BB′, CC′, etc., and if they are arranged within the two circles in the following manner: AA′BB′CC′DD′ and EE′FF′GG′, then, if chromosomes ABCD are attracted to the same pole as EFG in one cell, they will be so attracted in all normal pollen mother cells, and A′B′C′D′E′F′G′ will pass to the other pole. Only two chromosome complexes will be formed, and these will be the same as the complexes which united in the first place to form the plant. Lethal factors will make it impossible for any but a combination of the two complexes to survive, and hence, a permanently heterozygous species will result.

17. Irregularities in the zigzag arrangement of chromosomes at metaphase, such as are described in the text, will result in abnormally built complexes. Some of these will have eight chromosomes, and others only six. Still others will receive the normal number of chromosomes, in spite of the irregularity, but will nevertheless be incomplete, in that while they carry both members of one chromosome pair, they will lack entirely a representative of one of the other pairs. Of all these sorts of complex, the only ones likely successfully to function in reproduction are those with eight chromosomes.

18. Many complexes which are formed as the result of irregularity, however, will have the normal number of chromosomes, and a complete set of them; but from one to three of these will not belong normally to the complexes in which they find themselves, but rather to the opposite ones. Thus, for example, an irregular arrangement of a certain sort will yield the following complexes: A′BCDEFG′ and AB′C′D′E′F′G. The members of the homologous pairs AA′ and GG′ have gotten into the wrong complex. This phenomenon I have called "chromosome exchange"

to distinguish it from the ordinary kind of crossing over, which may perhaps also occur in these plants.

19. (a) When pollen grains arising from the last-mentioned type of irregularity are included among those used in crosses with other species, and the sperm developed from them function, the plants derived from these particular zygotes will exhibit crossing over with reference to the characters dependent upon genes which lie in the chromosomes which have crossed over into the wrong complex.

(b) In cases of self-pollination, plants arising through the functioning of such sperm with normal eggs will possess one or more chromosomes in duplicate, and if these are not the ones which possess zygote lethals, the plants should survive, and display certain new characters dependent upon recessive genes in those chromosomes which are now in the homozygous condition, as well as the lack of certain characters that are due to dominant genes in the corresponding chromosomes which are absent. The extent to which such plants will vary from the normal will depend upon the number of chromosomes which have exchanged places, and the consequent number of recessive characters which have therefore become evident, as well as the number of dominant characters which are lost. In rare cases, the plants may vary to such an extent as to warrant calling them mutants.

(c) Where two plants of the same species, but not traceable to a common self-pollinated ancestor are crossed, the chances of variations occurring when such a sperm fertilizes an egg will be considerably reduced, inasmuch as the occurrence and accumulation of gene mutations in the corresponding chromosomes of the two strains will have rendered these chromosomes less identical than in cases where both parents have had a relatively recent common origin.

20. The chances of a given irregularity, and hence of a given morphological variation occurring are very small.

21. *Oe. biennis sulfurea* is identical with *Oe. biennis* in its cytological behavior, so that the *sulfurea* factor has undoubtedly arisen as the result of a gene mutation.

In conclusion, it is a pleasure to acknowledge the kindness of Professor B. M. DAVIS, in permitting me to collect material in his experimental garden. To members of the botanical staff of the MARINE BIOLOGICAL LABORATORY, Woods Hole, Massachusetts, I am also indebted for laboratory facilities placed at my disposal during the summers of 1923 and 1924.

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DYNAMICS OF THE WAXY GENE IN MAIZE. I. THE CARBOHYDRATE RESERVES IN ENDOSPERM AND POLLEN[1]

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INTRODUCTION

The fact that the waxy factor in maize manifests its action in the pollen, that is, immediately following the reduction divisions, has suggested to us that favorable material might be found here for a study of the gene as a dynamic unit.

Up to the present time there has been no marked tendency in genetical research to go behind the superficial effects of the hereditary unit. There appear to be two main reasons for this; the elucidation of many important problems has not required it, and, secondly, in complex organisms such studies are beset with very considerable experimental difficulties. But in proving the genes the material bases of heredity and the controlling agents in development some of the most fundamental questions in biology have been transferred to these entities. Studies relating to the arrangement of genes in the chromosomes and the mechanics of their distribution have been highly successful, but the methods employed have not served in any commensurate degree to reveal the intimate nature of the factors or the manner in which these units function in ontogeny. In contemporary genetics the genes are essentially static elements. The advancement of our knowledge of them from obscure germinal constituents recognizable only by some admittedly partial effect on a somatic feature to dynamic units of known physico-chemical properties is an objective whose possibilities of attainment we may well examine.

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There seems to be little hope, at this stage, of applying the methods of the constitutional chemist to this problem, so far as the particulate hereditary material itself is concerned. The isolation in pure form of any gene would appear to present insuperable difficulties, especially in view of the probable instability of such substances and the manifest difficulty of establishing their identity *in vitro*. The possibilities which may be involved here cannot yet be given serious consideration. It does not appear probable, moreover, that a study of the whole nucleus by the chemical methods at present available can shed much light on the properties the geneticist has found this organ to possess. In such a problem as the one in hand the chemist can render unique service, but his efforts will be more fruitful, in our opinion, if directed upon gene action rather than upon the genes themselves.

Since direct methods of inquiry are precluded, we are forced to study the gene through the effects it produces during development. The distribution of the hereditary units has been followed by the external modifications which they occasion in the organism, but little knowledge of the nature of these elements has been gained thereby. We must go deeper and attempt to trace out the chain of physiological processes which links the superficial changes to the hereditary factor. The nature of these reactions should afford some clues to the dynamic properties of the gene itself.

It is a common observation that a single factor may cause a variety of changes and the criticism might be made at the outset that this method of attack can never lead to a satisfactory comprehension of gene action on account of the sheer multiplicity of these effects. Even though under diverse circumstances, internal and external, different results are found, these do not necessarily imply different kinds of activity on the part of the gene. It may be that the primary effects of a single hereditary unit are definitely restricted and the manifold changes that we see may ensue as these basic activities impinge upon various other processes. To use a crude analogy we may compare the possible action of a gene to that of a pathogenic organism which, through the liberation of a specific toxin, causes a variety of alterations in the highly differentiated host which it invades.

Assuming then that many of the effects occasioned by the presence of a gene are secondary, our problem resolves itself into recognizing the primary ones and gaining access to them. In this we encounter real difficulties. The external changes which have led to the recognition of the factor may give no clue to the nature of the underlying processes. For various reasons, moreover, these processes may not be amenable to investigation. It will be seen, however, when we review the progress that has already been made in some lines that promise of success in such efforts is not wholly lacking.

In approaching this subject it should be borne in mind, however, that, unless we accept BATESON'S presence-and-absence hypothesis, an allelomorphic difference cannot be taken as indicative of the total action of a gene. On the assumption that both the dominant and recessive conditions have germinal representatives at the locus in question, our results would not bear upon the nature of either of these genes as whole units but upon the difference between them. We would be measuring, not what is added to the residual inheritance by one gene, but the differential action of two. On this latter basis a knowledge of the complete action of a gene would be possible only in so far as mutations were available, revealing all the potentialities of the original factor. Investigations such as the present may at best, therefore, reveal only a partial action of the gene. Similar studies on the members of a multiple allelomorphic series might afford a more complete picture.

The waxy character in maize, with which the present investigation deals, was first described by COLLINS (1909), who noted that it involved a new type of endosperm. The physical properties of the non-floury portions of this tissue resembled those of a hard wax, hence the name. In inheritance waxy behaves as a simple recessive to the non-waxy or normal condition. It was discovered by WEATHERWAX (1922) that the endosperm reserves form a reddish-brown compound with iodine, a fact which serves to distinguish this race from all other known varieties of maize.

Recent investigations by DEMEREC (1924), BRINK and MACGILLIVRAY (1924) and LONGLEY (1924) have demonstrated the action of the waxy gene in the pollen also. Whereas starch grains giving the typical blue reaction with iodine are formed in the pollen of other races, the granule in the pollen of waxy plants, although not staining as quickly, assume a reddish color when similarly treated. The most significant fact in this connection is that segregating plants produce the two kinds of pollen in equal proportions. This proves the activity of the non-waxy-waxy allelomorphs in the male gametophyte. In the vegetative tissues of the plant, granules staining red with iodine have not been found, but blue-staining starch may be plentiful.

From the developmental standpoint the gametophyte in flowering plants is vastly simpler than the highly differentiated sporophyte. We are probably safe in assuming, in view of the facts now at hand, that the potentialities of the waxy gene, so far as fundamental changes in carbohydrate metabolism are concerned, at least, are as fully revealed in the pollen as in the diploid plant. If this is true, we may consider the waxy gene as conditioning primarily certain fundamental chemical reactions which are not dependent upon a high degree of differentiation of the tissues in which they occur. That is, the waxy gene appears to be concerned with basic metabolic processes and we may treat it without reference to the complications which surround its action in the much specialized sporophyte. This circumstance is of first importance in a study of the dynamics of the factor. At the same time the parallel effect in the seed furnishes an abundance of material for experimental investigation.

Previous studies have shown that the waxy gene occasions a modification in the kind of carbohydrate reserves laid down in the endosperm and pollen. We have found striking differences in enzyme activity in non-waxy and waxy plants, also, and it is to these two phases of the subject that our current investigations mainly relate. In this paper it is proposed to set forth the evidence regarding the above-mentioned carbohydrate reserves as a basis for the fuller consideration that can be given when the facts regarding enzyme differences are also examined.

We wish to acknowledge the aid rendered in these studies by Miss KATHLEEN DIETRICH, who has prepared the accompanying drawings and coöperated in certain of the experiments. Our thanks are due Miss MARGARET COLE for the faithful reproduction in plate 1 of the colors given by the non-waxy and waxy starches when treated with iodine.

CHEMICAL COMPOSITION OF POLLEN AND SEED

It has been stated by WEATHERWAX (1922) that the reserve material in the endosperm of the waxy maize consists of a single carbohydrate, erythrodextrin. The fact that this tissue gives a red color with the iodine-potassium-iodide solution does, indeed, suggest that dextrins may be the preponderating constituents, but chemical analyses do not bear this out. As table 1 shows, the principal reserve carbohydrate in the endosperm of waxy maize is starch. There is less than 2 percent of dextrins. These facts are of considerable interest.

The non-waxy seed used in our analyses came from a commercial variety of white dent known as Silver King, while the waxy grain was taken from an unrelated stock being used in pedigree-culture work. This diversity in origin might be considered objectionable when our aim is to distinguish between the effects of the non-waxy and waxy allelomorphs only, but the results of the analyses are so clear-cut in regard to the relative proportions of the three main classes of carbohydrates under consideration that this criticism is not important.

Three lots of non-waxy and waxy maize were analyzed. The first pair of samples comprised the dry seed; the second, grain which had been soaked in water for 24 hours at room temperature. The third lot consisted of three-day-old seedlings in which the plumules were one-quarter to one-half inch long. Since hydrolysis of the polysaccharides present is one of the chief reactions attending germination, it was believed that comparative analyses of this series should reveal any important differences in amount of the more readily available dextrins, if such difference existed in non-waxy and waxy maize. Only sugars were determined on the dry seed, but in the other two samples the amounts of starch and dextrin were measured also.

Except for the sugar determinations, in which the improvements suggested by SHAFFER and HARTMANN (1921) were adopted, the standard analytical methods approved by the ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS were used. It may assist in the interpretation of the results if the more important steps involved in the separation of the three main groups of carbohydrates are briefly indicated. Weighed amounts of the finely ground oven-dried material are extracted for 24 hours with water-free, alcohol-free ether to remove the fatty substances. Extraction of the residue with 90 percent alcohol boiled under a reflux condenser for 75 minutes brings the sugars into solution. The dextrins are then removed by a further extraction for 3 hours with 10 percent alcohol at room temperature and determined as dextrose after hydrolysis with 1 percent HCl. The sugar-free dextrin-free residue is then boiled, cooled to 38°C and digested with saliva until the solution gives no color with iodine, fresh saliva being added if necessary to complete the hydrolysis. The starch is likewise measured by the amount of dextrose formed. Separation of the dextrins and starch in the sugar-free material is dependent, therefore, upon the difference in solubility of these substances in 10 percent alcohol at ordinary temperatures. Let us now consider the relative amounts of these carbohydrates in the two classes of seed. The results are shown in table 1.

TABLE 1

Content of starch, dextrin and sugars in waxy and non-waxy maize. Values for carbohydrates are percentages based on dry weight.

SAMPLE	MOISTURE DETERMINATION		STARCH		DEXTRIN		SUGARS		
	Dry matter	Percent moisture	After 10 per-cent alcohol extraction	After water extraction	Extracted with 10 percent alcohol	Extracted with water	Reducing	Sucrose	Total
1¹ Non-waxy	91.94	8.06	. .	.. .	. . . .		0.18	1.71	1.89
Waxy	92.69	7.31	. . .	.	. . . .	. . .	0.18	1.81	1.99
2² Non-waxy	94.57	5.43	57.85	62.83	0.90	1.28	0.28	1.14	1.42
Waxy	. . . .	. . . .	59.93	62.68	1.87	1.53	0.31	1.39	1.70
3³ Non-waxy	. . . .	. .	62.97	. . .	0.87	. .	0.79	0.94	1.73
Waxy	94.71	5.29	60.18	. . .	1.61	. . .	0.80	1.78	2.72

1¹ = dry seed; 2² = soaked 24 hours; 3³ = germinated (plumules ¼ inch to ½ inch long)

It is quite evident that these results are not consistent with the hypothesis that the principal reserve materials in waxy endosperm are dextrins. In sample 2, soaked for 24 hours, the content of "starch" in the waxy type is as great as that in the common variety and amounts to nearly 60 percent of the total substance. Furthermore, the amount of material separating out in the starch fraction of the waxy sample is over thirty times that appearing as dextrin. According to these analyses the waxy seed before germination contains about twice as much dextrin as the starchy, but the absolute quantities are very small in both cases, amounting to less than two percent. It is a point worthy of note that the samples taken during early germination do not show, in either case, an increase in dextrin content. With the possible exception of the dry seed, the waxy sort appears to contain somewhat larger amounts of sucrose than the non-waxy type.

Thinking that the low yield of dextrin from the variety staining red with iodine might be due to incomplete extraction, the process was continued for an equivalent period of time. No further dextrin was recovered from the non-waxy sample and the amount yielded by the waxy residue was so very small as to be negligible. Prolonged extraction of the sample soaked 24 hours with water in place of 10 percent alcohol only slightly altered the proportions of starch and dextrin, as the table shows.

Pollen was collected for analysis from four sets of plants, two of which were sister progenies derived, respectively, from the non-waxy and waxy seeds borne on a single self-pollinated ear. These latter stocks were designated R 1a and R 1b. In progeny R 1a the homozygous non-waxy plants only were used as the source of this class of pollen, the heterozygotes being excluded in the field on the basis of the color reaction of their pollen with iodine. We have found this test entirely reliable and very convenient for making this separation. Pollen was also gathered from a group of Rice popcorn plants and an unrelated waxy progeny.

Immediately following collection the pollen was dried in a vacuum oven for 24 hours at 40°C and then placed in a desiccator over concentrated sulfuric acid.

The results of the analyses are given in table 2. Starch determinations were made on the last-mentioned lots of pollen only. While waxy pollen does not show a blue reaction with iodine, it does contain about the same amount of starch as the non-waxy sample. The dextrin content of the two sorts of pollen is very similar, namely, about 2.25 percent, or roughly one-sixth the amount of starch, About one-half the total carbohydrates in the pollen, exclusive of celluloses, exists in the form of starch in both these classes of maize.

TABLE 2

Percentage composition of non-waxy and waxy pollen with respect to sugars, dextrin and starch. Results are calculated to moisture-free basis.

DESIGNATION	STARCH	DEXTRIN	REDUCING SUGARS	SUCROSE	TOTAL SUGARS
Non-waxy (Rice pop)	16.28	2.29	2.55	7.43	9.98
Waxy (unrelated)	14.96	2.16	1.95	8.93	10.88
Non-waxy (R 1a)	....	2.19	2.20	8.59	10.79
Waxy (R 1b)	.....	2.33	0.95	11.32	12 27
Average non-waxy	16.28	2 25	2.37	7.81	10.27
Waxy	14.96	2.24	1.05	10.52	11.57

So far as the present results go, there appears to be a constant difference in the two kinds of pollen in the proportions of reducing sugars and sucrose, the non-waxy type containing less of the latter substance and more of the former than the waxy pollen. This may be of significance in relation to invertase activity.

The significant thing in these proximate analyses of the carbohydrates is the close similarity in the composition of the non-waxy and waxy types of maize. Both varieties contain about 60 percent of starch in the seed and about 15 percent in the pollen. Nor is there a very considerable difference in the relatively small amounts of dextrin present. On the basis of these quantitative determinations the principal reserve material in the pollen and endosperm of the waxy race is to be considered a starch and not a dextrin. One must be cautious, however, in classifying these polysaccharides about which so little is known as chemical individuals. Starches and dextrins may possibly merge into each other by imperceptible degrees and the distinction between certain of them may be rather arbitrary. For purposes of quantitative estimation, however, solubility in 10 percent alcohol or in water at ordinary temperatures is considered the distinguishing feature and on this basis we have no other recourse but to place the principal reserve material of waxy endosperm and pollen among the starches.

On the other hand the waxy reserves differ from ordinary starches in regard to a property which has been considered one of the most distinctive characteristics of this group of carbohydrates, namely, the ability to form a blue compound with iodine. There can be no question that the waxy starch differs from the common type. With a view of studying the nature of this difference we have prepared the two starches in pure form.

PREPARATION OF STARCHES IN PURE FORM FROM ENDOSPERM AND POLLEN

In the preparation of starch from the endosperm, whole corn was soaked for 12 hours at 60°C in a solution of sulfurous acid of specific gravity 1.007. The liquid was then run off and the softened grain beaten to a pulp in a large mortar. The embryos, the bran and any other coarser particles were then removed by washing on a wire sieve. The starchy portion was again crushed and kneaded through muslin of coarse mesh and the starch allowed to settle. This crude starch was purified by thoroughly agitating it in a 0.40 percent solution of sodium hydrate until the milky liquid assumed a greenish-yellow color. This addition of alkali assists in the removal of proteins and the remaining oil. After sufficient treatment with sodium hydroxide the separated starch and proteins were allowed to deposit and the supernatant solution was drawn off. The impure starch was then agitated with water and allowed to stand 20 minutes to permit the coagulated nitrogenous compounds to settle. The starch in suspension above was siphoned off and allowed to settle. After rewashing several times it was thoroughly dried. This method yields a refined product in satisfactory amounts.

It is much more difficult to prepare a clean sample of starch from the pollen. The starch grains are much smaller and the relatively large amounts of fatty substances are difficult to remove. A fairly pure product was obtained, however, in the following way. Five grams of pollen were extracted for 5 days with ether at room temperatures followed by absolute alcohol for 7 days. The alcohol was brought to 35°C and held for several hours during this period. After the alcohol was filtered off the pollen was mixed with clean quartz sand and a little water and ground in a mortar for about 30 minutes. The resulting paste was poured on a fine muslin cloth and kneaded in water until little further material passed the cloth. The suspension was allowed to settle for an hour, the supernatant liquid containing most of the starch then decanted off, leaving considerable sand behind. The starch was then collected by centrifuging for 5 minutes at 1800 r.p.m., but much fine sand was also thrown down. This mixture was partially separated by repeated centrifugation, but very small quartz particles remained as the principal impurity after drying.

FORM OF THE STARCH GRAINS FROM ENDOSPERM AND POLLEN

In order to secure strictly comparable material for these studies the waxy and non-waxy grains on a series of backcrossed ears were separated and the starches prepared from them according to the method described above. Figure 1 is a photomicrograph of the starch grains from the non-waxy seeds. The waxy starch is shown in figure 2. Both these preparations were lightly stained with iodine. It is a noteworthy fact that endosperm reserves of the waxy variety are organized in granular form, as is the case in common maize. There is about the same variation in size of starch grain in the two types, but there appear to be small differences in contour.

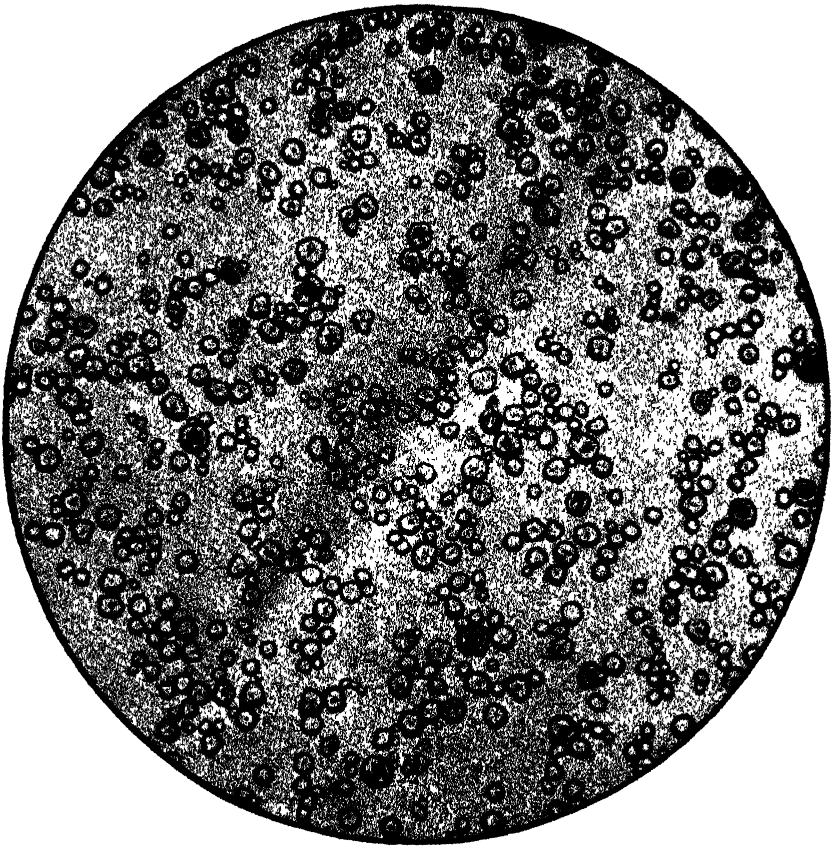

FIGURE 1.—Photomicrograph of the starch grains from non-waxy maize endosperm, lightly stained with iodine. Magnification ca. 250 diameters.

In general, the waxy grains are somewhat more angular than those of the other type. In both, the hilum is centric, but the non-waxy starch appears to be more finely fissured. It is possible that these relatively small morphological differences are due in part to the weak alkali used during preparation. There is a marked differential effect on the two classes of starch grains when treated with strong alkali or concentrated chloral hydrate, the shape of the waxy granules becoming quickly distorted. The swelling properties in water differ also.

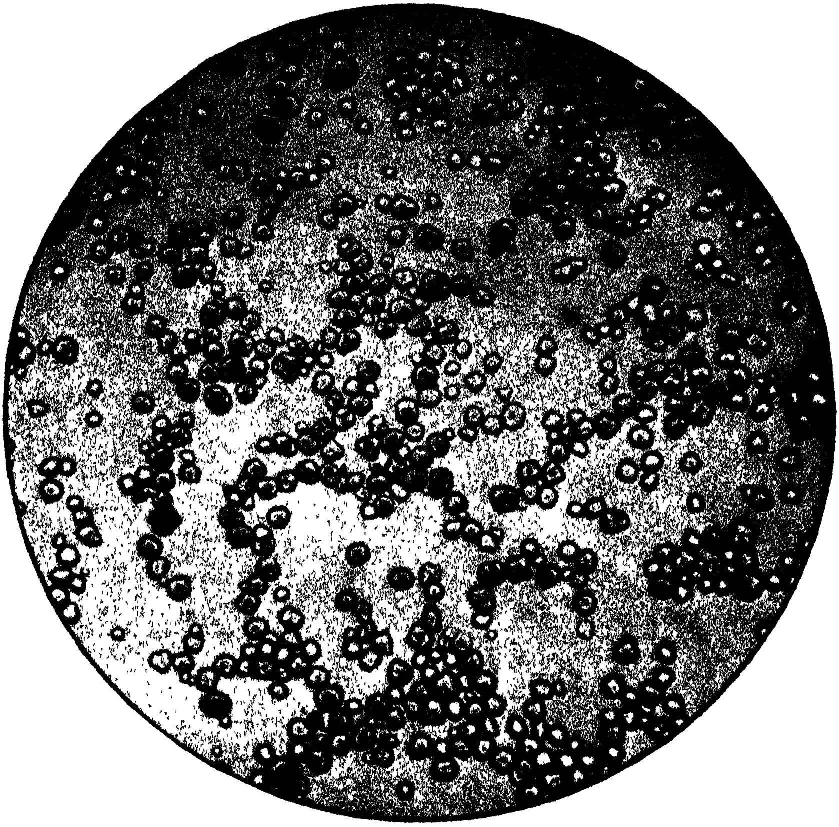

FIGURE 2.—Photomicrograph of the starch grains from waxy maize endosperm, lightly stained with iodine. Magnification ca. 250 diameters.

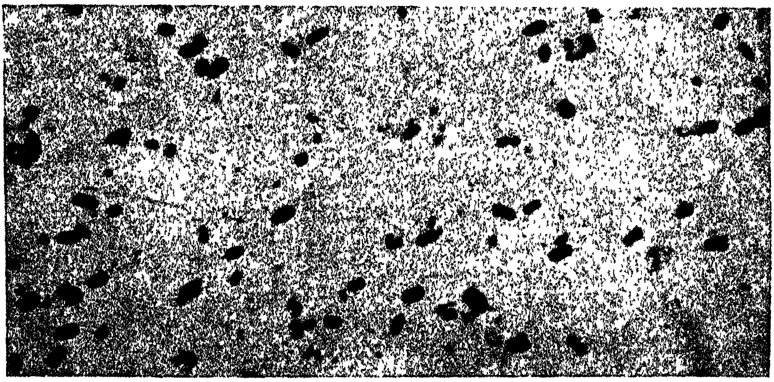

FIGURE 3.—Photomicrograph of starch grains from the pollen of non-waxy maize, heavily stained with iodine. Magnification ca. 900 diameters.

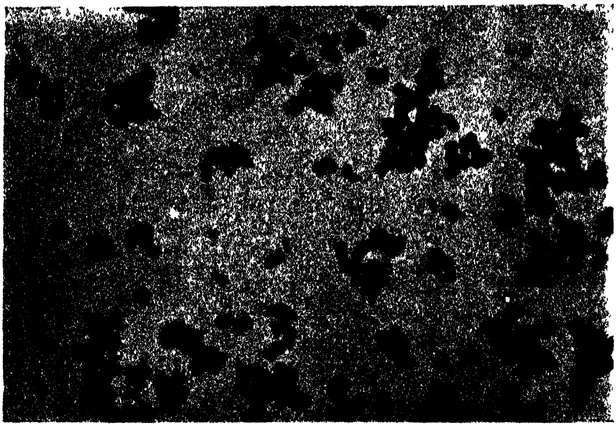

FIGURE 4.—Photomicrograph of starch grains from the pollen of waxy maize, heavily stained with iodine. Magnification ca. 900 diameters.

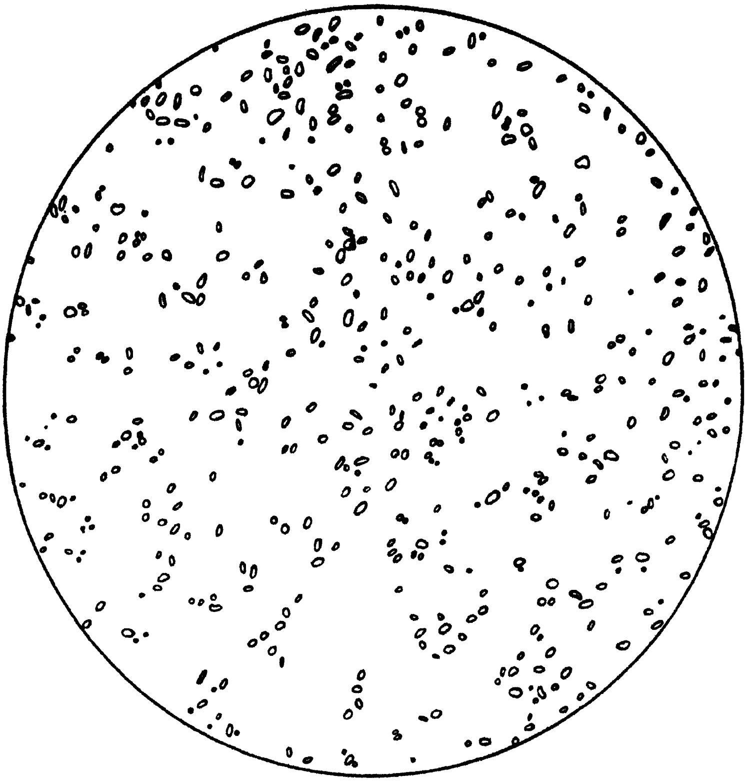

FIGURE 5.—Drawing of starch grains from the pollen of non-waxy maize. Magnification ca. 600 diameters.

In the pollen the starch grains are much smaller, seldom measuring more than $4\mu \times 1.5\mu$ through their main axes. As shown in figure 3 the granules from non-waxy pollen are quite regularly ovoid in shape. In waxy pollen, on the other hand, the grains are often dumb-bell-shaped, although, as may be seen from the photograph reproduced as figure 4, their form is less regular than in the former case. The differences between the starches in the two types of pollen are more clearly brought out in the drawings shown in figures 5 and 6. There is unquestionably a real morphological difference in the pollen starch grains of the waxy and non-waxy races of maize.

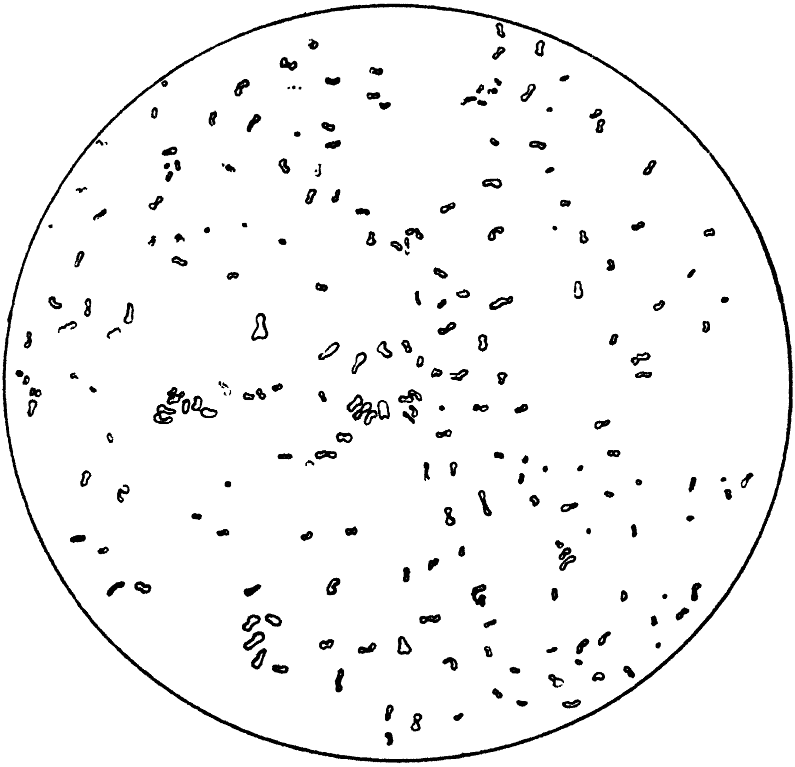

FIGURE 6.—Drawing of starch grains from the pollen of waxy maize. Magnification ca. 600 diameters.

It is noteworthy that the waxy granules do not show the etching characteristic of the starch grains in the sugary race of maize and the wrinkled variety of the pea.

When examined under polarized light the starch grains from the endosperm of non-waxy and waxy maize show no marked differences. In

LEGEND FOR PLATE 1

FIGURE A.—Color reaction of aqueous suspensions of gelatinized pollen and endosperm starch from non-waxy maize with iodine-potassium-iodide.

FIGURE B.—Color reaction of aqueous suspensions of gelatinized pollen and endosperm starch from waxy maize with iodine-potassium-iodide.

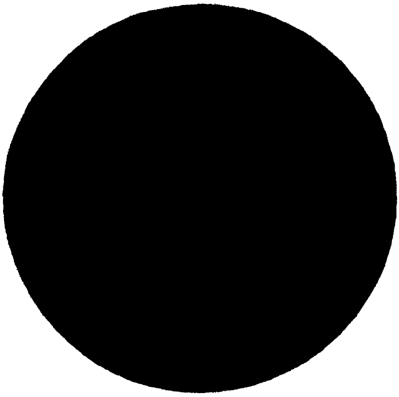

FIGURE A

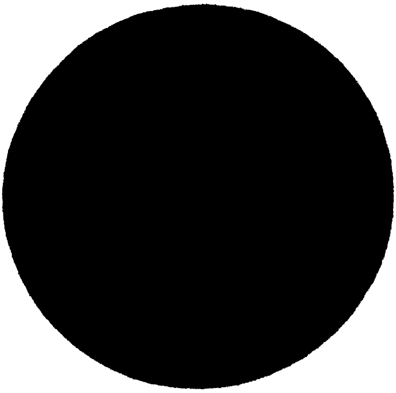

FIGURE B

both types the figure is usually centric and the lines are commonly at right angles to each other. The bands are possibly more clear-cut in the case of the waxy granules.

COLOR REACTIONS OF THE ENDOSPERM AND POLLEN STARCHES WITH IODINE

The most striking difference in the starches from waxy and non-waxy seeds is shown on treatment with the common iodine reagent (1.0 percent potassium iodide, 0.3 percent iodine in aqueous solution). Starch from the endosperm of ordinary maize, in common with that from nearly every other source, produces an intense blue color under these conditions. This result is so generally characteristic that it has come to be known as the "starch reaction." The starch grains in the endosperm of waxy maize, however, form a reddish-brown compound with iodine. The solutions prepared by boiling 0.1 percent of these two starches with water likewise react very differently to this test. If to 10 cc samples 5 drops of the above reagent be added, the ordinary starch gives a color described by RIDGWAY (1912) as Helvetia Blue, whereas that of the waxy starch is Rood's Violet. The concentration of starch and iodine may be widely varied without obliterating the difference.

The starch grains from the pollen of common maize also form a deep blue compound immediately on being treated with iodine. This reaction is not obtained with starch from waxy pollen. These latter do not stain at all when the reagent is first applied, but if the solution is concentrated by evaporation of the water and renewed from time to time, a reddish color is formed. The identification of non-waxy and waxy pollen (DEMEREC 1924; BRINK and MACGILLIVRAY 1924) rests upon this differential color reaction of the respective types of starch grains with iodine. If small amounts of the pollen starches are boiled and a few drops of iodine added to the suspensions after cooling, the same color reactions are obtained as with the corresponding endosperm reserves, namely, Helvetia Blue and Rood's Violet. In plate 1, figure A, is shown the color reaction with iodine of starch from the pollen and endosperm of non-waxy maize; figure B illustrates the color obtained on similarly treating he pollen and endosperm starches of waxy maize.

The identity of the color reactions with iodine and the similarity shown in rate of hydrolysis with amylase, to be discussed below, make it seem probable that there is a close correspondence in chemical composition between the pollen and endosperm starches in the two respective classes of maize. Their resemblance with respect to this property is good evidence for the view that the same fundamental carbohydrate transformations occur in the pollen, which is an independent and relatively simple, binucleate structure, as in the endosperm, where the accessory conditions are more complex. As we have pointed out above, this circumstance should make the basic physiological action of the waxy gene more accessible to investigation.

COMPOSITION OF THE STARCH GRAIN

Agreement regarding the composition of the starch grain has by no means been reached. The literature pertaining to the subject is very large and the reader who desires an exhaustive treatment is referred to the summaries found in CZAPEK (1913) and especially ABDERHALDEN (1923). We shall limit ourselves here to a few of the more pertinent contributions.

The idea that the starch grain is not composed of a single homogeneous substance has received support from numerous investigations. NÄGELI (1858) distinguished two main constituents, granulose, the more soluble portion responsible for the blue color with iodine, and starch cellulose which, he considered, formed the skeleton of the grain. MAQUENNE and ROUX (1903, 1905) contend that the principal constituent of the starch grain is amylose, a substance which gives a blue reaction with iodine and exists in two polymeric forms, one of which is soluble in hot water and the other in superheated steam. A second substance, which they term amylopectin, is mainly responsible for the viscosity of starch pastes. It swells in hot water but does not dissolve, and is incompletely hydrolyzed to sugars with diastase. MEYER (1895) had earlier described two similar substances which he termed β-amylose and α-amylose, respectively. BIEDERMANN (1920) distinguishes a third substance called amylocellulose, which remains after treatment of amylopectin with salivary diastase. LING and NANJI (1923) have also found a substance in the starch of wheat, barley and rice, that behaves like a hemicellulose. In contrast with these views, BOURQUELOT (1887) assumes that the starch grain is composed of a large number of closely related carbohydrates.

SHERMAN and BAKER (1916) have shown that when 1 percent raw starch is gelatinized in water at 85°C and centrifuged at the rate of 2400 r.p.m. for 30 minutes it is separated into a lighter, clearer, more limpid layer containing the more soluble constituent (β-amylose of MEYER, amylose of MAQUENNE and ROUX) and a heavier more viscous layer containing the less soluble component (α-amylose of MEYER, amylopectin of MAQUENNE and ROUX). The presence of 0.001 M NaCl is necessary in order to effect the partition. SHERMAN and BAKER found that potato starch treated in this way yielded approximately 20 percent of β-amylose, to adopt MEYER's terminology. Some important differences in the properties of the two fractions were established. It required from 13 to 18 times as much of the twice-washed residual gel (crude α-amylose) to produce a given intensity of blue color with iodine as was required of the β-amylose. The β-amylose was found to be unstable, rapidly changing to the insoluble form even when stored in a refrigerator. With malt amylose the β-fraction is more readily hydrolyzed to compounds giving no reaction with iodine than is the α-fraction.

The starches prepared from waxy and non-waxy maize differ very greatly in the proportions of α- and β-amylose which they contain. After some preliminary attempts to separate these constituents, the following method, which differs from that of SHERMAN and BAKER only in that no effort was made to purify the products obtained from the first centrifugation, was adopted. Two-gram samples of each of the starches were heated in 100 cc of water for 10 minutes at 85°C. After adding enough tenth-normal NaCl to make the suspensions 0.002 M, they were centrifuged for 30 minutes at 2400 r.p.m. The non-waxy type of starch under this treatment separates into two distinct layers, as SHERMAN and BAKER found with potato-starch suspensions, the upper one being relatively clear and the lower very thick and slimy.

TABLE 3

Relative amounts of crude α- and β-amylose in the endosperm starches from non-waxy and waxy maize.

	TEST 1		TEST 2	
	Non-waxy	Waxy	Non-waxy	Waxy
Total yield in grams of crude β-amylose from 2 grams of starch	0.1587	1.2317	0.1587	1.1934
Percent of crude β-amylose	8.0	61.6	8.0	59.7
Percent of crude α-amylose by difference	92.0	38.4	92.0	40.3

The starch from the waxy type of grain as shown in figure 7 gives a quite different picture in the centrifuge tube. While the line of demarkation is nearly as well defined, the relative volumes occupied by the two layers and their appearance are very different. The upper layer in the waxy tube is not as deep as that in the non-waxy one, but in the former the concentration of the more soluble β-amylose is so much greater that it gives this stratum a heavy opalescent appearance. Moreover, the lower layer in the waxy tube is less viscous than the α-amylose fraction from the non-waxy starch.

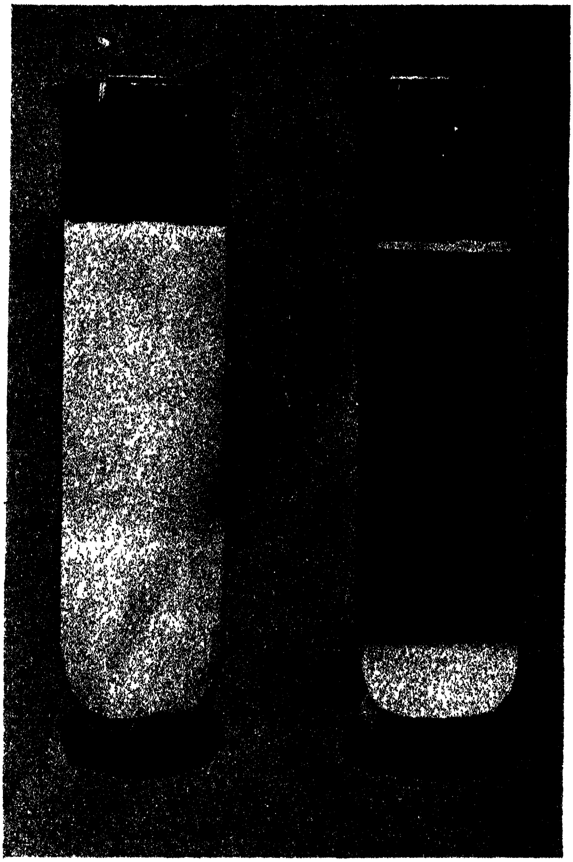

FIGURE 7.—Photographs of one percent suspensions of waxy starch (left) and non-waxy starch (right) after centrifugation for 30 minutes at 2400 r.p.m. The arrows point to the junctions of the layers of α- and β-amylose.

The supernatant layers containing the β-fractions were pipetted off and their volumes measured. To determine the amounts of starch present, 50 cc samples were concentrated on a steam bath and dried over night in an oven at 100°C and weighed. Table 3 shows the relative proportions of crude α- and β-amylose in the two kinds of starches.

The results of the two tests made are in close agreement.[2] The waxy type of starch contains about seven and one-half times as much of the more soluble constituent as the sample prepared from the non-waxy seeds borne on the same ears.

It is not to be inferred, however, that the α-fractions or the β-fractions are chemically identical in the two cases and that the differences in the two kinds of starches are explicable on the assumption that they are composed of the same two substances but in different proportions. When tested with iodine, both constituents of the waxy starch show the color described by RIDGWAY (1912) as Rood's Violet, whereas the preparations from the non-waxy starch stain the familiar blue. It is a significant fact in support of the view that the main reserve material of the waxy endosperm is a starch and not a dextrin, that the waxy-type granules comprise two substances which separate under the action of centrifugal force in the same way as the α- and β-amyloses of the common starches. But, as indicated by their iodine reactions and their behavior under the action of hydrolytic agents discussed below, there is some fundamental difference in these amyloses from non-waxy and waxy starches, on which the present experiment sheds no light.

RATE OF HYDROLYSIS OF NON-WAXY AND WAXY STARCH WITH HCl AND AMYLASE

Experiments were made to determine if the starches from non-waxy and waxy seeds were hydrolyzed at different rates under the action of dilute HCl and of salivary amylase. As a measure of the rate of digestion, the time required for the solution to reach the stage at which no color was given with iodine was taken.

The procedure with an acid hydrolysis was briefly as follows: 2-gram samples of each of the two sorts of starch were weighed out and boiled with 100 cc of water for 5 minutes. The volumes were brought to 96 cc and the solutions transferred to 500 cc Erlenmeyer flasks. These were fitted with reflux condensers and placed in a water bath. After bringing the liquid to boiling temperature, 4 cc of 25 percent HCl solution were added to each flask, giving a concentration of hydrochloric acid of 1 percent. A delivery tube was arranged so that samples could be forced

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[2] The two determinations were not carried out at the same time, so the identical values for total yield of β-amylose from the non-waxy type in tests 1 and 2 are not to be explained by assuming that the same sample was weighed twice. There was either a mistake in our records or the case is an unusual coincidence. But even if the results of the one or the other test are thrown out, the main point is clear.

TABLE 4

Rate of hydrolysis of non-waxy and waxy starch paste with 1 percent HCl.

TEST 1			TEST 2		
Time in minutes required for hydrolysis		Ratio of times, waxy = 1	Time in minutes required for hydrolysis		Ratio of times, waxy = 1
Non-waxy	Waxy		Non-waxy	Waxy	
45	30	1.50 : 1	53	32	1.65 : 1

out periodically by means of air pressure without opening the flasks. This made it possible to maintain a constant temperature and prevented changes in concentration of the reagents. During the early stages of the digestion color tests against a background of white tile were made at 10-minute intervals and as the reaction approached completion, at 5-minute and 2-minute periods.

The results in table 4 show that the waxy starch is hydrolyzed to compounds giving no color with iodine, about one and one-half times as rapidly as the non-waxy starch. The differences in time in tests 1 and 2 are due in part to the difficulty of distinguishing the end point exactly, although the limits within which one can be sure this falls are relatively narrow. It is clear from the results obtained in these experiments that the waxy starch is less resistant to acid hydrolysis than the non-waxy starch.

The difference in rate of hydrolysis of the two kinds of starch with salivary amylase is very much greater than with acid. In fact, the disparity is so large that it is difficult to arrange a satisfactory comparative test. The amount of saliva necessary to carry the digestion of non-waxy starch to completion will hydrolyze the same quantity of waxy starch so rapidly that the rate cannot be measured. If the saliva is reduced sufficiently to enable the hydrolysis of the waxy sample to be accurately followed, digestion of the non-waxy sample is only partial, considerable starch remaining even after some hours.

After several fruitless attempts to measure the rates of these reactions by using different amounts of enzyme for the two kinds of starches, two experiments were made which were considered fairly satisfactory so far as opportunity to time the reaction and sharpness of end point were concerned. It was not found possible, however, to get as accurate results as in the tests on acid hydrolysis. The starch was boiled 5 minutes and diluted to 0.25 percent. The digestions were carried out on a water bath at 40°C. In the first experiment 5 cc of undiluted saliva were added to

50 cc of the solution of non-waxy starch. For the hydrolysis of the waxy starch the saliva was first diluted 1 to 4 and only 2.5 cc were used, added to the same volume of solution. The latter sample, therefore, contained one-eighth the amount of enzyme in the former. If we assume, as a first approximation, that the rate of hydrolysis is a linear function of concentration of enzyme, the time required for digestion of the non-waxy starch should be multiplied by 8 to make it comparable with that for the waxy sample. The result given in table 5 under test 1 indicates that the waxy starch is hydrolyzed over one hundred times as rapidly with salivary amylase as is the non-waxy starch.

TABLE 5

Rate of hydrolysis of non-waxy and waxy starch pastes with salivary amylase.

TEST 1			TEST 2		
Time in minutes required for hydrolysis		Ratio of times, waxy = 1	Time in minutes required for hydrolysis		Ratio of times, waxy = 1
Non-waxy	Waxy		Non-waxy	Waxy	
45×8*	3	120 : 1	16×16*	4	64 : 1

* Correction factors for differences in concentration of enzyme.

In test 2 the same amounts of starch were employed, but 16 times as much enzyme was used in the non-waxy sample as in the waxy. The end point with the solution of non-waxy starch was taken somewhat earlier than in the first test, namely, when the color with iodine was passing from olive to yellow. A little starch undoubtedly remains at this stage, but the rate of disappearance of the last traces is so low that a later reading must be rather arbitrary. The ratio of times in this test, when corrected for the difference in amount of enzyme, was found to be 64 : 1.

A remarkable difference in ease of hydrolysis of non-waxy and waxy starch with saliva is thus demonstrated. It seems probable that this difference is shown by both the α and β components. We have not tested these substrates with saliva, but some other experiments with amylase preparations from non-waxy and waxy seedlings on the two β-amyloses, respectively, although not made with this particular point in mind at the time, shed some light on the question. The concentrations of starch in the solutions tested were only approximately known, but it appeared that the waxy β-amylose was hydrolyzed about 3 times as rapidly as the corresponding substance from non-waxy starch. While the amount of the difference may be subject to revision it seems clear that the waxy β-

amylose is broken down more rapidly. A difference of this order, however, will not explain the great disparity in rates of hydrolysis of the whole starches. This is to be attributed mainly, we believe, to the dissimilarity in digestibility of the α-amyloses, this fraction from waxy starch being more readily hydrolyzed.

Experiments on the rate of hydrolysis of the α-amyloses themselves are unsatisfactory, since, with ordinary amounts of enzyme the reaction does not go to completion. The presence of an undigested residue obscures any earlier end point that might be taken.

Tests on rate of hydrolysis of the pollen starches with salivary amylase show that the waxy type is more readily digested than the non-waxy sort. Due to the impurities (principally fine quartz sand) in our preparations, it was not possible to put these experiments on a satisfactory quantitative basis. The difference in times required for disappearance of color with iodine in the two cases were so large, however, that there can be no doubt of the fact that the red-staining starch is broken down much more speedily than that from non-waxy pollen.

DIGESTION OF THE ENDOSPERM RESERVES DURING SEEDLING DEVELOPMENT AND RATE OF GROWTH IN THE SPROUT

In view of the relative ease with which waxy starch is hydrolyzed by amylase, it might be expected that during germination the endosperm reserves of this form as compared with those of the non-waxy type would be more quickly digested, and that the seedlings would make a more rapid growth. This does not prove to be the case, however. The endosperm of the non-waxy variety is utilized nearly as rapidly as that in waxy maize, and the two classes of sprouts make about equal gains in weight during the seedling stage.

In the experiments which led to these conclusions non-waxy and waxy seedlings were grown in the dark under conditions of controlled temperature and soil moisture and the changes in dry weight of the seed residue and the sprouts followed. In the first test it was found, on collecting the initial samples, that about 40 percent of the stored materials in the endosperm had already been removed. The experiment was therefore repeated, collections being made frequently enough to follow the whole course of seedling development. The seed used came from 4 carefully selected ears which had resulted from backcrossing heterozygous non-waxy plants with the waxy type. The grains from the butts and tips of the ears and any obviously defective individuals were discarded. Eight lots of 55 seeds each of the non-waxy and waxy types, respectively, were counted out

TABLE 6

Decrease in weight of endosperm of non-waxy and waxy grains during seedling growth. The values given are relative weights of the respective residues, the check samples being taken as 100.

AGE OF SEEDLINGS IN DAYS	WEIGHT OF SEED RESIDUES	
	Non-waxy	Waxy
0	100.0	100.0
1	100.4	98.0
2	93.7	93.0
3	78.2	81.3
4	68.0	70.1
6	39.5	34.4
8	23.4	21.4
9	21.5	18.0

and by substitutions of smaller grains for larger or *vice versa*, each sample was adjusted to weigh 12.0 grams. After soaking in water for 24 hours the seeds were planted in clean quartz sand approximately 50 percent saturated with moisture in galvanized iron boxes 13 inches by 4 inches by 4 inches. In each of these containers two 55-seed samples, a non-waxy and a waxy, were planted, the groups being separated by a movable center strip running from end to end. The boxes were weighed and placed in a darkened incubator set at 30°C. Throughout the course of the experiment the water lost by evaporation was replaced daily. Arrangement of the test in this fashion made the conditions under which the two classes of seed were grown closely comparable.

Samples consisting of the two lots of seedlings in a box were collected at the intervals given in table 6. After the sand was washed out of the roots with as little loss of tissue as possible, the sprouts and the residues of the seeds were separated at once. While the plants are fresh this operation can be performed cleanly, the adhesion of the embryo to the seed residue being very slight. The separate tissues were then dried at 100°C for at least 15 hours and weighed.

Up to the third day all the seeds or seedlings were included in the weighed samples, but thereafter the ungerminated grains were discarded. Five lots comprised fewer than 50 individuals, the lowest number being 41. In computing the values given in tables 6 and 7, corrections for variations in number were made by adjusting the weights to a basis of 50 seedlings.

The data given in table 6 are plotted in figure 8. It is evident from the graph that during the early stages of seedling growth the non-waxy and waxy endosperms decrease in weight at the same rate. During the latter

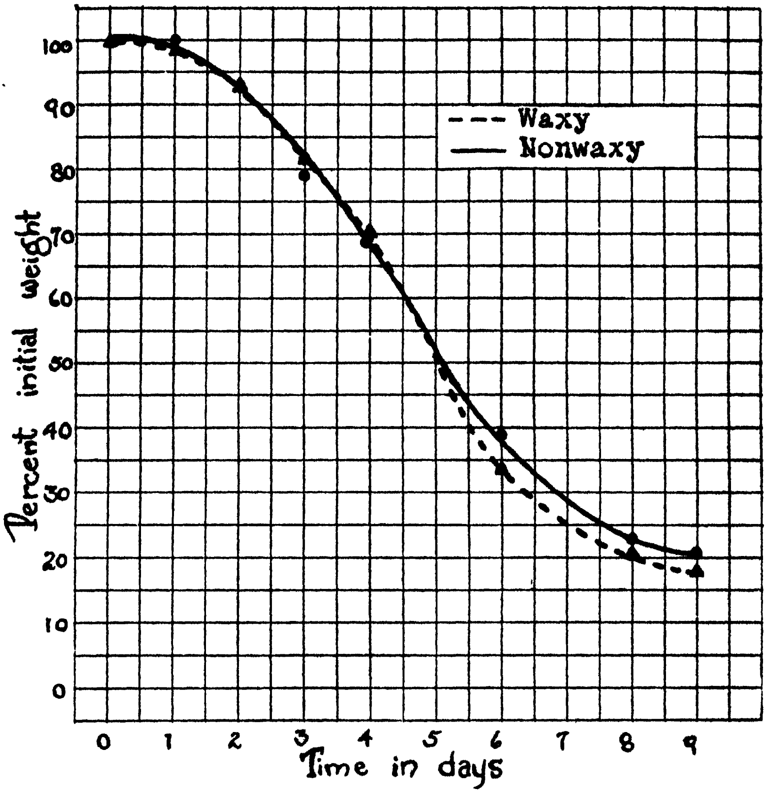

FIGURE 8.—Decrease in weight of the seed residues (endosperm and pericarp) during early growth of non-waxy and waxy maize plants in darkness. The curves represent the percentages of the inital amount of material remaining as the development of the seedlings progresses.

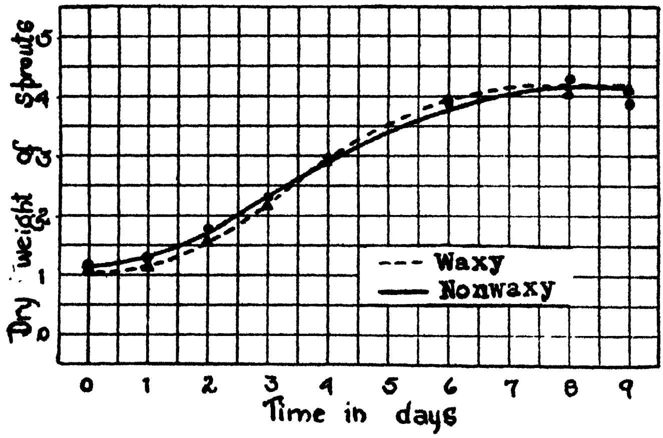

FIGURE 9.—Increase in dry weight of sprouts of non-waxy and waxy plants during the seedling stage. The plants were grown in darkness.

TABLE 7

Weight in grams of non-waxy and waxy sprouts during seedling growth. The values are adjusted to a basis of 50 individuals.

AGE OF SEEDLINGS IN DAYS	WEIGHT OF SPROUTS IN GRAMS	
	Non-waxy	Waxy
0	1.18	1.05
1	1.34	1.12
2	1.73	1.56
3	2.36	2.27
4	2.90	2.84
6	3.86	3.96
8	4.37	4.07
9	3.77	4.14

half of the cycle, however, it would appear that the waxy reserves decrease more rapidly, although the difference in mass of undigested material is never more than 5.1 percent and decreases to 3.5 percent on the ninth day. The results obtained in test 1, which are not reported in detail here on account of their incompleteness, agree in showing that from the fourth to the eighth day the waxy reserves are utilized somewhat more rapidly than those in the non-waxy seed. There may be, therefore, a significant though small difference in the rapidity with which the two kinds of starches are digested. In test 1, which was carried to the twelfth day, no further decrease in weight of the seed residues occurred after ten days. At this time the two types were alike in retaining about 15 percent of the original substance. It is probable, therefore, that both go to the same "end point."

During the seedling period the sprouts increase in weight nearly fourfold. As table 7 and figure 9 show, the difference between the non-waxy and waxy starches in this regard is probably not significant. The results obtained in test 1, as far as they go, also support this conclusion.

In view of the ease with which the waxy starch is hydrolyzed with amylase *in vitro*, the very close correspondence in rate of digestion of the non-waxy and waxy endosperms during seedling development calls for an explanation. Quantitative studies which we have made on the amylases produced during germination show that the amounts of this enzyme in the waxy seedlings is distinctly less than that formed by the non-waxy type. An interesting compensating feature is thus revealed, which, from the standpoint of our study on the nature of the waxy gene, may be highly significant. In waxy seed we have an easily digested reserve starch, but a relatively small production of amylase, whereas the starch of the non-waxy variety shows a greater resistance to hydrolysis, but is accompanied by a larger amount of enzyme. It seems probable that these effects of the waxy factor bear a definite genetic relation to each other.

In the field, waxy plants attain the same development as their non-waxy sibs, which demonstrates that the waxy gene does not occasion the absence of any particular essential for the manufacture of protoplasm. The present results on weight of sprouts indicate that the time relations of the ultimate synthetic processes involved in growth are not appreciably altered. So far, therefore, as the general economy of the plant is concerned, the waxy gene does not cause any significant changes. If the waxy factor acted upon a variety of metabolic processes in the seedling, we might expect this to be reflected in an altered rate of growth. There is direct evidence that the gene modifies certain carbohydrate transformations. Where these may become limiting, as in pollen-tube growth (cf. BRINK 1925), we do find a change in the rate at which the individual develops. While it cannot be definitely concluded that there are no other effects, the evidence favors the view that the waxy gene is limited in its sphere of action to changes in carbohydrate metabolism.

DISCUSSION

The present investigations have been undertaken in the belief that we may gain some knowledge of the dynamic properties of genes by studying the physiological changes occasioned in an organism by the substitution of one allelomorph for another. The idea is nearly as old as genetics itself, although the work based upon it is not extensive. The early results on color inheritance in plants and animals aroused considerable speculation as to the chemical bases for the differences found. The Mendelian relations were often simple, and sufficient was known regarding the constitution of certain pigments to engender the hope that the hereditary phenomena might be interpreted in terms of chemical transformations. Workers of the English school have made some notable advances and have opened up a field, which, though beset with many difficulties, should yield further important results to the investigator with the requisite training in chemistry and genetics.

In Antirrhinum the formula *YYII* has been assigned to the ivory type and *YYii* to the yellow-flowered form, on the basis of their breeding behavior. Plants lacking *Y* are albinos. WHELDALE (1916) has shown that the pigments concerned here are two flavones, apigenin and luteolin. The yellow variety contains both these compounds, but the plants of the ivory class produce only apigenin. Albinos contain no flavone and this is attributed to the absence of the *Y* factor. The *I* gene may be considered as responsible for the production of a substance which inhibits the formation of luteolin, or, since the latter compound is more highly oxidized than apigenin, *I* may be a reducing agent. Evidence is presented which indicates that each of these two pigments is built up of phloroglucin and an oxybenzoic acid. On treatment with caustic alkali apigenin forms P-oxybenzoic acid, whereas luteolin yields protocatechuic acid. The *I* gene may operate, therefore, to prevent the formation of this latter compound.

The existence of complementary factors for flower color has suggested the hypothesis that one of the germinal elements concerned gives rise to an oxidase which, acting on a chromogen formed by the other gene, produces pigment. This idea, however, has not been substantiated. WHELDALE (1916), working with Lathyrus and Matthiola, obtained qualitative evidence for flavones in both the white races which give color when crossed. Likewise oxidases proved to be present in both. WHELDALE considers, however, that the critical tests have not been made. A difference may yet exist in these white races in regard to a specific flavone concerned in the formation of the pigment. In regard to the oxidases, it is pointed out that these substances are probably not completely wanting in any plant, and if means were found of differentiating between various oxidases, a significant difference in this regard in the white strains might be revealed.

The survey made by KEEBLE and ARMSTRONG (1912) also showed oxidases in both the white-flowered sweet peas carrying complementary factors for color. Their results in general are strongly suggestive, nevertheless, of a close relation between these enzymes and pigment formation. They further found that the intact corollas of dominant-white *Primula sinensis* plants gave no reaction for oxidase. After treatment with dilute hydrocyanic acid, however, a positive test was obtained. These facts led the authors to conclude that "The results confirm the Mendelian hypothesis that dominant-white flowers owe their lack of pigment to the presence of an inhibitor of pigment formation."

Using the chemical evidence adduced by GORTNER (1911, 1912), ONSLOW (1915) and others, WRIGHT (1917) has elaborated an ingenious hypothesis to account for the color variations in mammals. The chemical evidence indicates that the pigments of the eye, fur and skin of mammals, known collectively as melanins, are formed from tyrosin or related products of protein metabolism through the agency of oxidizing enzymes. It seems probable that there are various chromogens, those used being dependent on the character of the enzymes present. The hereditary differences in color are due to differences in the kind or amount of enzyme involved in the reactions attending pigment production. WRIGHT assumes two enzymes, I and II, color depending on the rate of production and potency of these.

> "Enzyme I is essential to the production of any color, but by itself only produces yellow. Enzyme II is supplementary to enzyme I, producing no effect by itself. The compound enzyme, I-II, produces a darker kind of pigment than enzyme I alone, viz., sepia. Enzyme I-II is also more efficient than enzyme I in another way. It produces sepia pigment even when enzyme I is at too low a potency to produce any yellow by itself. Above the level at which enzyme I produces effects, the two enzymes, I and I-II, compete in the oxidation of chromogen. Chromogen which is oxidized by enzyme I to yellow pigment is incapable of further oxidation to black. In the mixture the presence of the relatively pale yellow colors serves mainly to dilute the color of the hair. This production of yellow reduces the amount of dark pigment and the apparent intensity of color, both by reducing the amount of enzyme I which can unite with II to form the enzyme for black production and also by using up chromogen which would otherwise become black."

WRIGHT interprets a very large array of breeding facts on this hypothesis, but points out that

> "Any such assignment of physiological effects to factors is at present to be taken mainly as a means of visualizing their action in our ignorance of the real physiology."

While WRIGHT'S theory of gene action utilizes the available chemical facts, it rests mainly upon the hereditary relationships. It is clear that much further knowledge of the processes involved in the production of melanin pigments must be obtained before it can be measured against adequate chemical evidence.

In view of the direct effect of the waxy factor in the pollen of maize and the ease with which starch may be prepared from the grain and amylases extracted from the seedlings, it would seem that particularly favorable material is offered here for a study of the dynamics of a gene. The similarity of the reserve starch produced in waxy pollen and waxy endosperm makes it seem probable that the gene functions in the simple gametophytic structure as it does in the green plant in so far as carbohydrate changes are concerned, at least. There are three lines of evidence pointing to the conclusion that the main carbohydrate reserve in the pollen and endosperm of waxy maize is the same substance. In quantitative chemical analyses the chief storage product in each case is found to separate out in the starch fraction and microscopic examination shows starch grains in both tissues. The starches from these two sources, when prepared in pure form and dissolved in water, give the same color reaction with iodine, namely, Rood's Violet. And thirdly, they are alike in being more readily hydrolyzed by amylase than non-waxy starch. The assumption is reasonably well founded, therefore, that the waxy gene governs the same series of reactions in the pollen as in the developing endosperm. The fact that these reactions occur in the simple binucleate gametophyte leads us to the view that they are primary effects of the waxy gene.

It has been our aim in the present investigations to determine so far as possible the difference in the nature of the reserve materials of the non-waxy and waxy races of maize as the first step in the analysis of the action of the waxy gene. Proximate chemical analysis shows at once that the view of WEATHERWAX (1922) that the reserve material of the waxy endosperm is erythrodextrin is not well founded. The term erythrodextrin, so far as we have been able to learn, was first used by BRÜCKE (1872) who applied it to the product obtained on roasting starch. Contending that this treatment gives a mixture of dextrins, LINTNER and DÜLL (1893), by repeated fractional precipitation with alcohol, claim to have prepared a pure erythrodextrin. This substance which gave a reddish-brown color with iodine, as its name suggests, was *readily soluble in water.* The reserve carbohydrate characteristic of waxy maize gives the red-brown color reaction by reflected light, but we find that it is not soluble in water or in 10 percent alcohol, the reagent employed in standard methods of analysis to extract dextrins from mixtures containing starch. BRÜCKE'S (1872) preparation was probably what we know today as "commercial dextrin." This substance is also readily soluble in water. The waxy reserve, therefore, does not conform to either of the substances previously described as erythrodextrin. ARTHUR MEYER (1886), whom WEATHERWAX (1922) quotes as giving the only comprehensive account of this polysaccharide, does not use the term erythrodextrin. We must not be misled into thinking that the waxy reserve is necessarily a dextrin simply because it gives a red color with iodine; the animal physiologists have long recognized glycogen, which forms a reddish-brown compound with iodine, as having the properties of a starch. There seem to be no good reasons either chemical or historical, therefore, for calling the reserves of waxy endosperm erythrodextrin.

We prefer to look upon this material as a starch. If it must have a name we may with propriety follow MEYER (1886) in calling it "red-staining starch." This red-staining starch is characteristic of the endosperm of certain other cereals also. GRIS (1860) found it in *Oryza sativa* var. *glutinosa*, and MEYER (1886) established its presence in *Panicum miliaceum* var. *canditum glutinosum* and *Sorghum vulgare glutinosum.* It has also been found in another grass, *Coix lachryma-jobi* (LONGLEY 1924). From the account given by TADOKORO and SATO (1923), it seems very probable that the endosperm reserves of glutinous rice are identical with those of waxy maize. Indeed, it appears likely that we have the same substance in all five species.

MEYER (1886) attempted an explanation of the occurrence of starch grains which stained red with iodine. He considered that the starch granules of glutinous rice and glutinous sorghum were composed of small amounts of starch, staining blue with iodine, some amylodextrin, giving a red color with iodine, and a third substance present in the largest amount which he called dextrin. This latter substance gave a brown color with iodine. According to the author the amylodextrin arises through the action of a weak ferment on the blue-staining starch deposited in the grain. TANAKA (1907) denies the presence of other compounds in the starch grains of glutinous rice and considers the red coloration with iodine a characteristic of the starch itself. TADOKORO and SATO (1923) have made a study of some colloidal properties of glutinous rice starch and conclude that it may differ from common starch only in the degree of polymerization.

In our own work we have examined the composition of the starches of non-waxy and waxy maize as revealed by centrifuging suspensions at high speeds, which treatment, as SHERMAN and BAKER (1916) found, serves to separate in a crude way the two principal constituents of the grain. Further, we have compared the rates at which these starches are broken down to compounds giving no color with iodine under the action of hydrolytic agents. Observations on the color reactions of the whole starches and their components after centrifugation have also been made. Although we cannot draw definite conclusions on the basis of the facts at hand, we may indicate some possible differences between the non-waxy and waxy starches. We shall consider the evidence regarding color with iodine first.

As we have already pointed out the α- and β-amyloses prepared from the two starches give the same color reactions as suspensions of the respective whole starches. The criticism might be made that the assumption of color by the relatively insoluble α-amyloses is due to their being contaminated with β-amylose. The fact, however, that the α-amylose fractions tenaciously retain their power of staining under the action of amylase and hydrochloric acid which rapidly hydrolyze the β-amyloses, leads us to think that the two components of each of the respective types of starch are really alike in this regard. Whatever this fundamental difference in organization is which causes waxy starch to form a red compound with iodine, it characterizes both the fractions separated by centrifugation.

Harrison (1911) has made a study of the color reactions between starch and iodine from the colloidal standpoint. He reaches the conclusion that the red and blue colors are due to differences in degree of dispersion of the iodine particles. Any agency, such as alcohol, potassium iodide or heat, which tends to bring the iodine into true solution, changes the color in the direction of blue to red. According to this view the ultimate particles of starch from waxy maize must be smaller than those of the non-waxy type, thus presenting a greater surface on which the iodine is distributed. This might indicate a lower degree of polymerization of the red starch, but it must be admitted that the present knowledge of the color relations of starch-iodine solutions is rather unsatisfactory.

The nature of the difference between non-waxy and waxy maize starch is a problem the ultimate solution of which must wait upon a knowledge of the constitution of the molecules involved. A few years ago this statement would have implied an abandonment of the problem, for it seemed then that the structure of the starch molecule was scarcely accessible to analysis. But in the preface to the fourth edition of his monograph on "The carbohydrates and the glucosides," Armstrong (1924) remarks: "The outstanding advance, a very great one, recorded in this edition, is that relating to the complex carbohydrates, inulin, starch and cellulose." The work of Irvine (1923) and his school, Pringsheim (1923), Ling and Nanji (1923) and others has given an insight into this puzzling question which presages for the near future a knowledge of the structure of the starch molecule far more precise than could have been hoped for by the organic chemist of a decade ago, who found the methods which had proved so fruitful in other directions of little avail with this complex polysaccharide. Naturally, in a field where the significant developments are so recent there are many points of controversy, but certain general deductions may be drawn, which, if they do not find direct application to our problem in its present stage, at least indicate the lines along which certain phases of it may eventually be solved.

As hydrolysis with a strong acid shows, starch is composed entirely of glucose residues. The problem the constitutional chemist has sought to answer is the number and arrangement of these residues in the basal unit, the molecule, and the manner in which these units are polymerized. Irvine (1923) who has brought one particular method of inquiry, the methylation process, to a high degree of usefulness considers that the basal unit in starch is a tri-amylose $(C_6H_{10}O_5)_3$ and has deduced a structural formula in keeping with the results of the reactions studied.

LING and NANJI (1923), recognizing the heterogeneous nature of the starch granule, have first separated its two main constituents which they term polymerized amylose and amylopectin. In the starch of wheat, barley, rice and arrowroot they find that these substances are alike and are present in the ratio of 2 to 1. The starch grain may also contain varying amounts of another substance, probably a hemicellulose. LING and NANJI find that amylose and amylopectin yield different products on being hydrolyzed with appropriate enzymes. Thus, polymerized amylose when treated with precipitated barley diastase at 50° is hydrolyzed readily and quantitatively to maltose, whereas amylopectin under these conditions is scarcely attacked. The authors consider that the properties of amylose are best accommodated on the assumption that it is an α-hexa-amylose in which carbons 1 and 6 of the six glucose residues are united through oxygen atoms in the form of a ring compound. There are 6 α-linkings in this molecule the significance of which is that it may be hydrolyzed by maltase which attacks α-glucosides but not the stereoisomeric β-glucosides towards which emulsin has a specific action. The basal unit of amylopectin is considered to be a phosphoric ester of $\alpha\beta$ hexa-amylose. SAMEC (1914) previously demonstrated the presence of phosphorus as being in organic union with starch. LING and NANJI find that when amylopectin is treated at 70° with precipitated malt diastase previously heated to 70° a hexatriose $(C_{18}H_{32}O_{16})$ is formed. With emulsin this triose produces glucose and maltose, whereas glucose and iso-maltose are formed when it is hydrolyzed with yeast maltase or malt diastase. This indicates both α-linkings and β-linkings. Four of the former and two of the latter are postulated in the cyclic formula containing 6 glucose residues assigned to amylopectin.

Our experiments show that on heating 1 percent suspensions of non waxy and waxy starch at 85° for 10 minutes and centrifuging at 2400 r.p.m. for 30 minutes, the starch separates into two layers, the upper one containing crude β-amylose and the lower one crude α-amylose. With this treatment the non-waxy starch yields about 8 percent of the former substance and the waxy starch about 60 percent. The yield of β-amylose from the non-waxy starch is about one-half that obtained by SHERMAN and BAKER (1916) on treating potato starch in this way.

LING and NANJI (1923), working with potato starch also, which, according to them, consists almost entirely of amylose and amylopectin, appear to have obtained much larger yields of amylose by their methods. The different procedure we have followed makes it impossible, therefore, to judge with much assurance whether the large difference in proportions of α-amylose and β-amylose in non-waxy and waxy maize starch is indicative of a departure in one (or both) cases from the 2 : 1 ratio of amylose and amylopectin which LING and NANJI have found to hold with a number of other common starches. Nevertheless, the much larger amount of the more soluble constituent we have obtained from waxy starch cannot be without significance. It is indicative of a difference either in the abovementioned ratio or in the proportion of hemicellulose in the starch grains.

We have shown that the starch from waxy maize is hydrolyzed about twice as quickly by 1 percent HCl as that from the non-waxy type and over sixty times as rapidly by salivary amylase. Furthermore, the precipitated amylases from both non-waxy and waxy seedlings digest β-amylose from waxy starch more rapidly than the corresponding fraction from non-waxy starch. These facts imply some profound differences either in the ultimate starch molecules themselves or in the manner in which these basal units are polymerized. In the absence of evidence concerning structure in these particular starches we cannot do more than indicate the various possibilities that may be involved. Knowledge of the constitution of the polysaccharides has now been advanced to the stage where this may be done in a rather definite way and we have the assurance that methods are available which put the solution of this phase of our problem within reach of the carbohydrate chemist. The starches from non-waxy and waxy maize probably differ in one or more of the following ways:

(1) In the structure of the basal unit in regard to
    (a) Number of hexose residues
    (b) Manner in which these hexose residues are linked, that is, the particular carbons involved and the stereoisomeric nature of the linkings.
(2) In the degree of polymerization with reference to
    (a) Number of molecules condensed together
    (b) Manner in which the molecules are condensed.

It is fully realized that the most important facts relating to the effect of the waxy gene on the nature of the starch reserves are still to be gained. We must look mainly to the constitutional chemist for these. Investigations on the structure of the starch molecule are now being pushed forward with vigor and the recent successes will undoubtedly encourage further workers to enter this field. We may look forward with some confidence, then, to a much extended body of chemical data which may serve as a basis for interpreting the dynamic action of the waxy gene.

When tested under closely controlled conditions we find that waxy seedlings develop in the dark at nearly the same rate as those of the non-waxy type. During the latter part of the seedling stage the waxy reserves appear to be digested somewhat more rapidly as compared with the non-waxy type, but the sprouts do not increase in weight at a greater rate. This situation prevails in spite of the fact that the waxy starch is hydrolyzed by amylase *in vitro* much more readily than the other sort. The explanation of this probably lies, as we have already suggested, in the nature or amount of the amylases accompanying these reserves. A possibility especially worthy of consideration is that the type of reserve originally synthesized in the endosperm is determined by the amylase present. Substrate and enzyme might consequently bear such a relation to each other that the reverse process, namely, hydrolysis, would be effected at the same rate in the two types. It may be, therefore, that the waxy gene conditions, primarily, the kind or amount of amylase formed. Enzyme studies now in progress should throw light on this question.

SUMMARY

1. The direct effect of the waxy gene in maize on the type of reserve carbohydrate formed in the pollen suggests that the primary physiological action of this factor may be relatively accessible to analysis. The evidence presented indicates that the chief reserves in pollen and endosperm are probably identical, which implies that the same carbohydrate transformations occur in the two parts. The fact that this effect is encountered in the simple binucleate pollen is strongly suggestive of its being the immediate result of the activity of the waxy gene. The important practical advantage in the parallel action in the development of the seed is the abundance of material the latter affords for investigation.

2. Proximate chemical analyses show that the principal reserve carbohydrate in waxy pollen and endosperm is a starch. The starches from these two sources when prepared in relatively pure form and dissolved in water give the same color reaction with iodine, namely, Rood's Violet (RIDGWAY). They are alike moreover in being more readily hydrolyzed by amylase than non-waxy starch. It is considered probable, therefore, that the starches from pollen and endosperm are chemically identical.

3. Application of the name erythrodextrin to this substance is not in accord with previous usage of the term and is not justified by the chemical

and physical properties of the material. Solubility reactions in water and in 10 percent alcohol, separation of suspensions on centrifugation into two fractions and behavior on hydrolysis with amylase and HCl show clearly that the substance is a starch.

4. In form the starch grains from the endosperm of non-waxy and waxy seeds show only slight differences. In the pollen the granules are much smaller and the two types show a characteristic difference in shape. The two types of starch cannot be distinguished on the basis of their appearance with polarized light.

5. On centrifuging dilute suspensions prepared by heating at 85° for 10 minutes the starches are separated into two layers, the upper one being clear and more limpid, the lower, more opaque and viscous. Following MEYER'S terminology, we have designated these fractions crude β-amylose and crude α-amylose, respectively. The non-waxy starch yields about 8 percent of β-amylose by this method, whereas about 60 percent was found in waxy starch.

6. The α- and β-fractions from each of the respective starches give the same color reactions with iodine. The α- and β-amyloses from waxy starch are more readily hydrolyzed by amylase than the corresponding portions from non-waxy starch. It is concluded, therefore, that the non-waxy and waxy starches differ in regard to both these components.

7. On HARRISON'S hypothesis the violet color of waxy starch with iodine might be considered as indicating a lower degree of polymerization than prevails in the common types of starch.

8. Waxy starch is hydrolyzed by boiling 1 percent HCl about one and one-half times as rapidly as non-waxy starch.

9. With salivary amylase the difference in rate of hydrolysis is very much greater. On account of incomplete digestion the end points are more difficult to read, but it appears that the waxy starch is broken down by this enzyme at least sixty times as rapidly as common maize starch.

10. Amylases prepared from both non-waxy and waxy seedlings also hydrolyze the waxy starch at a greater rate.

11. Some recent important advances in the chemistry of starch are noted in the text, which indicate that a satisfactory solution of the structure of the starch molecule, which has so long defied analysis, is now within reach. While no information is available at present bearing directly upon the difference in the constitution of non-waxy and waxy starch, the possibilities in this regard may be formulated in a fairly definite way on the basis of the chemical knowledge now at hand. A study of waxy starch

along the lines developed by IRVINE and his associates and LING and NANJI might yield results of very great interest to the geneticist and the carbohydrate chemist also.

12. Waxy seedlings grown in the dark develop sprouts at the same rate as their non-waxy sibs. In the former the seed residue appears to decrease in weight somewhat more rapidly in the later stages, but the difference is much smaller than might be expected in view of the great ease with which waxy starch is hydrolyzed by amylase *in vitro*. The suggestion is offered that the type of reserve synthesized in the endosperm is controlled by the kind or amount of amylase present and that substrate and enzyme may bear such a relation to each other that the reverse process, hydrolysis, may proceed at the same rate in the two types of seedlings. On this hypothesis the initial effect of the waxy gene relates to the type or amount of amylase formed.

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"OLD-GOLD" FLOWER COLOR, THE SECOND CASE OF INDEPENDENT INHERITANCE IN OENOTHERA[1]*

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INTRODUCTION

The evening primroses display, as sharply as do any other plants or any animals, a mosaic-like combination of alternatively inherited characteristics, such as provide in most other organisms the clearest examples of typical Mendelian inheritance. A list of the more striking of these alternative characters includes: (a) tall *versus* dwarf stems; (b) red *versus* green stems; (c) red-spotted *versus* unspotted green leaves; (d) flat leaves *versus* revolute leaves; (e) narrow petals *versus* broad petals; (f) yellow petals

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versus sulfur petals; (g) red hypanthium *versus* green hypanthium; (h) red or pink bud-cones *versus* green bud-cones; (i) long styles *versus* short styles. The first-mentioned member of each of these pairs is the dominant. In addition to these, there appear to be at least four recessive lethals paired with four corresponding dominant "vital" factors. Of all these factors and some others which are known but not here enumerated, only the short-styled form of *Oenothera brevistylis* has been found, heretofore, to be distributed in inheritance independently of the others.

The relation between the long styles of *Oenothera Lamarckiana* and the short styles of *brevistylis* may be considered a classic example of Mendelian inheritance, as this pair of contrasting characters was included by DE VRIES (1900) in the list of 11 examples cited in his original statement of the "law of disjunction of hybrids,"—one of the three announcements of the three independent rediscoveries of the Mendelian laws.

Were no lethal factors involved, each of the other character pairs mentioned above should likewise yield typical Mendelian phenomena when handled singly in the production of monohybrids; that is, allowing for errors of random sampling and deviations due to selective viability, etc., they would give uniform F_1 families of the dominant type; would split in F_2 families, following selfing of the F_1, into 3 dominants to 1 recessive; on backcrossing to the recessive, they would give 1 : 1 ratios of dominants to recessives; and reciprocal crosses would give identical results.

The wide-spread occurrence of lethal factors has so modified this program that the Oenotheras have long seemed an outstanding exception to the general applicability of the Mendelian scheme of heredity.

I have shown in previous papers (SHULL 1921a, b, 1923a, b) that this disturbing effect of the lethals is due to the fact that all of the above-mentioned factors, except the pair for long and short styles, are associated together in a single linkage group (group I) in which the lethals likewise occur. I have assumed that the linkage of these factors is the result of their inclusion in a single pair of chromosomes, but recent cytological studies of CLELAND (1922, 1923, 1924, 1925, 1926) indicate the existence of a possibly alternative mechanism for the production of linkage in the Oenotheras, and it seems best for the present to admit the possibility that linkage may result from chromosomal cohesions (synzeuxis), though I still think that the available evidence is more favorable to the view that linkage in the Oenotheras, as in other organisms, is based on the inclusion of the linked genes within a single chromosome pair (monozeuxis).

This new fundamental problem as to the physical basis of linkage in the Oenotheras adds to the interest and importance of further genetical studies in this group of plants, and gives special significance to any factors which may be found to lie outside the big linkage group I with which nearly all studies have heretofore been concerned.

The factor for old-gold flower color (mut. *vetaurea*) has been the first, after *brevistylis*, to fulfill this condition. One of the first families in which it appeared (2047) showed plants of the *Lamarckiana* type segregating 40 yellow : 13 old-gold. In 1922 and 1923 self-fertilized *Lamarckiana* plants in the line of direct descent from the family in which the original mutation occurred, yielded typical 3 : 1 ratios of yellow and *vetaurea*-flowered *Lamarckiana*, namely, (No. 2123) 35 Y : 8 V, and (No. 2220) 22 Y : 7 V. These 3 : 1 segregations in typical *Lamarckiana* families, have been paralleled, heretofore, only by the segregation to the short style of *brevistylis*, and show that the *vetaurea* factor is not in the same linkage group as the *Lamarckiana* lethals,—therefore, not in group I.

The fact that *brevistylis* was the only previously known character which behaved in this way, made it seem desirable to test very completely the genetical relations of the old-gold mutation. For this purpose it has been a very fortunate circumstance that the old-gold factor has no noticeable injurious effects on the reproductive processes. It is thus greatly superior to *brevistylis* for such a study, since *brevistylis*, because of the deformed and disadvantageously placed stigmas, can be used as a seed parent only with the greatest difficulty, though it produces abundant pollen, and is therefore a very satisfactory pollen parent.

The present paper gives a complete account of the breeding results with the old-gold factor, with the exception of a few recent crosses made in an attack on certain problems which are not yet ready for report.

ORIGIN OF THE OLD-GOLD MUTATION

As described in a previous paper (Shull 1921b) the old-gold mutation (mut. *vetaurea*) manifested itself in 1921 in two families (2047 and 2052), both of which had the same individual grandparents 188 (12)×187 (56), the parents having been two individuals, (30) and (54), in family 198. A portion of the network of cross-breedings from which these two families were derived is shown in figure 1. With exception of the year 1914, this network of descent has been continued in the same manner, with annual generations, since 1905, when it was begun with ten rosettes of *Oenothera Lamarckiana* collected at my request by Professor Hugo de Vries, in the field at Hilversum, Holland, from which he had secured the foundational material for his own cultures almost 20 years previously. Every plant used in this network has been a typical *Lamarckiana*, and every family in this series of cultures has had the usual composition of *Lamarckiana* families, nearly all of the offspring in each progeny being typical *Lamarckiana*, but usually associated with a small percentage of mutant types, the majority of which have been recognizable as 15-chromosome forms. Only mut. *funifolia* (SHULL 1921b), discovered in 1918, and mut. *vetaurea*, the subject of the present paper, have been demonstrably produced as the result of gene mutations, and I believe that these are the only new characters in Oenothera which have been certainly ascertained to have been the result of gene mutations, as distinguished from the process of unmasking recessive factors which have been kept hidden because of their linkage with lethal factors.

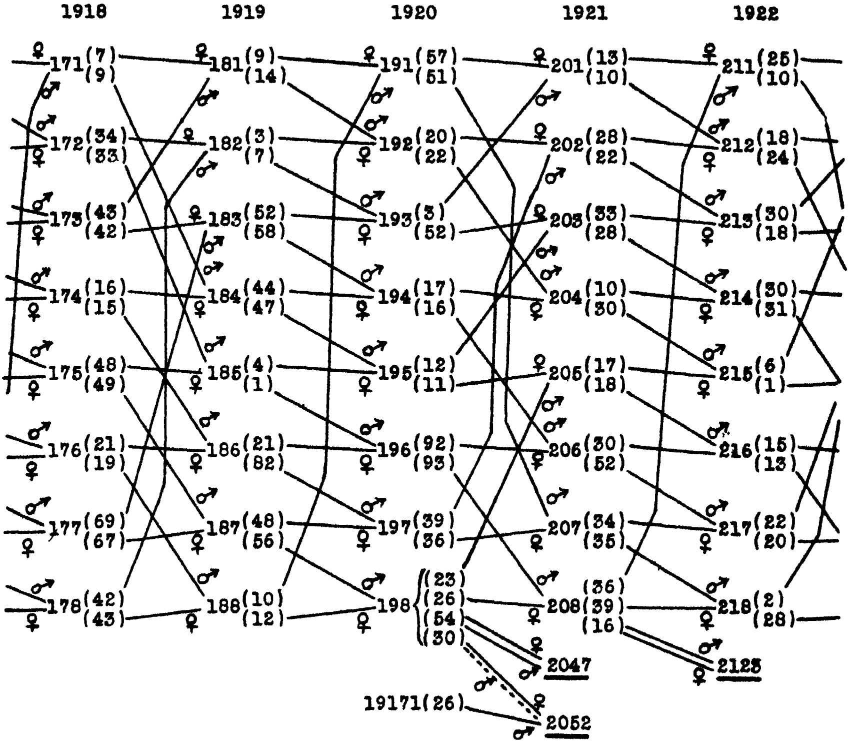


FIGURE 1.—Five generations of the network of descent in which "old-gold" originated as a gene-mutation. The three-figure numbers are family numbers, and the numbers in parenthesis are individual numbers. The year in which each generation was grown is indicated at the top of the corresponding column. Every individual represented was a typical *Lamarckiana* except 198(54) which was a green-coned mutant. Only in families 198 and 208 was the presence of old-gold as a hidden recessive type demonstrated. The pedigree numbers of families in which old-gold plants made their appearance *de novo* are underscored. The dotted line connecting 198(30) with 2052 represents an accidental self-fertilization, which alone made it possible for a *vetaurea* plant to appear in family 2052.

OLD-GOLD BREEDS TRUE

The first question which presents itself, on the discovery of a new characteristic in any organism, is whether it will breed true. Experience teaches that many such new forms are due to some accident of development that produces no effect whatever on the offspring; that others are apparently the result of complex combinations of genes which by chance only rarely recombine in the grouping necessary to produce again the characteristic in question; that some produce progenies which return in part to the parental type; and that still others segregate into one or more subordinate types. In table 1 are presented the results of 21 self-fertilizations of the old-gold type (mut. *vetaurea*), and of 14 crosses between two plants of the same type. The 35 families contained a total of 1772 old-gold, and none of any other type.

As will be seen in a later section, however, old-gold may segregate a subordinate type,—but only when it is not derived from a pure yellow (or old-gold) parentage.

OLD-GOLD RECESSIVE TO YELLOW

That the old-gold factor is recessive to yellow was indicated by the splitting of the first families observed, in 3 : 1 ratios of yellow to old-gold. The numerous crosses which have been made between forms having yellow flowers and forms with old-gold flowers, have always given only yellow flowers, except, of course, when the yellow-flowered form had been rendered heterozygous by a previous cross. Table 2 shows the results of 30 such crosses, the total F_1 yield of which was 1961 yellow-flowered individuals and none of any other color.

The most important series of crosses included in this table are those which involved as one of their parents an individual in families 201 to 208, for these are the families belonging to the network of descent shown in figure 1, in which the old-gold mutation occurred. The yellow-flowered parent in each of these nine families stood in a relation of cousinship of greater or less degree of closeness to the original mut. *vetaurea*. The fact that every one of these crosses yielded only yellow-flowered offspring in the F_1 demonstrates that the *vetaurea* factor was not present in the strain, hidden, as in some other Oenothera mutations,—for example *nanella* and *pervirens*,—by the presence of lethal factors linked with them. This renders it certain that the *vetaurea* factor originated as a gene mutation affecting one of the two gametes whose union gave rise to one of the parents of family 198, in which family, as we have seen, several individuals were demonstrably heterozygous for the *vetaurea* factor.

Table 1

Families which illustrate the true-breeding of mut. vetaurea.

PEDIGREE NUMBERS		SEEDS SOWN	PLANTS SECURED	PERCENTAGE GERMINATION	NUMBER FLOWERED, ALL VETAUREA
Parents	Family				
2052(10)	21108	300	63	21.00	45
2047(40)	21109	20	1	5.00	1
2047(92)	21113	184	127	69.02	105
2047(39) 2047(92)	21114	184	78	42.40	64
21108(19)	22147	300	79	26.33	3
21113(5)	22148	300	168	56.00	38
21159(63)	22216	300	123	41.00	36
21159(113)	22217	300	140	46.67	85
21108(19)	2389	300	66	22.00	44
2220(40)	2390	300	28	9.33	25
22148(69)	2391	261	119	45.59	55
2220(46) 22162(7)	2393	300	124	41.33	112
22167(41)	2398	300	183	61.00	86
22216(77)	23136	300	34	11.33	34
23135(5)	2486	300	172	57.33	107
23137(92)	24101	300	50	16.67	43
23137(92) 23137(80)	24102	300	212	70.67	143
23137(46) 23137(92)	24103	26	10	38.46	10
23137(91) 23137(92)	24104	300	70	23.33	54
2389(44) 23137(92)	24105	300	94	31.33	50
23137(92) 23144(30)	24112	300	85	28.33	61
23137(105)	24114	147	69	46.94	49
23137(46) 23137(105)	24115	24	7	29.17	5
23137(91) 23137(105)	24116	300	116	38.67	57
23137(46) 23137(80)	24119	35	7	20.00	3
23137(91) 23137(80)	24120	300	230	76.67	95
23137(91) 23137(63)	24124	300	217	72.33	133
23137(91) 23137(76)	24125	300	138	46.00	98
23137(91)	24126	300	56	18.67	31
23137(18)	24127	76	29	38.16	7
23137(46)	24128	154	53	34.42	15
23142(2)	24148	300	78	26.00	32
23142(2) 23144(30)	24149	245	37	15.10	30
2390(5)	24150	300	54	18.00	5
2391(43)	24151	25	12	48.00	11
Total					1772

TABLE 2

Families which show the recessiveness of vetaurea in the F_1 of crosses with homozygous yellow.

PEDIGREE NUMBERS			SEEDS SOWN	NUMBER OF PLANTS SECURED	PERCENTAGE GERMINATION	NUMBER FLOWERED, ALL YELLOW
Parents ♀	♂	Family				
2047(39)	2047(42)	21111	300	154	51.33	126
2047(40)	2046(159)	21115	134	42	31.34	40
2046(159)	2047(40)	21117	300	138	46.00	111
2047(39)	20195(1)	21118	168	64	38.10	60
2047(39)	20247(47)	21119	20	3	15.00	3
2047(39)	20183(36)	21123	323	115	35.60	113
2047(39)	20202(17)	21126	85	22	25.88	20
2047(39)	201(10)	21128	78	21	26.92	16
201(13)	2047(39)	21129	139	14	10 07	11
202(28)	2047(39)	21130	175	21	12.00	20
203(33)	2047(39)	21131	269	46	17.10	38
205(17)	2047(39)	21133	141	15	10.64	11
206(30)	2047(39)	21134	300	104	34.67	90
207(34)	2047(39)	21135	300	121	40.33	85
208(39)	2047(39)	21136	154	21	13.64	17
2047(39)	208(36)	21137	300	97	32.33	84
2047(39)	2031(13)	21138	161	97	60.25	94
2031(13)	2047(39)	21139	300	134	44.67	105
22148(69)	2295(43)	2392	169	103	60.95	90
22218(34)	22162(7)	23139	300	70	23.33	64
237(23)	23137(92)	24106	300	148	49.33	129
23138(44)	23137(92)	24110	300	94	31.33	87
23138(73)	23137(92)	24111	300	106	35.33	95
237(23)	23137(105)	24117	158	61	38.61	19
23138(73)	23137(105)	24118	300	91	30.33	52
237(23)	23137(80)	24121	259	201	77.61	143
23138(73)	23137(80)	24122	300	165	55.00	87
23137(46)	23138(73)	24131	34	3	8.82	3
23138(73)	23137(46)	24132	300	167	55.67	144
23137(46)	23138(73)	24133	49	4	8.16	4
Total						1961

SEGREGATION OF OLD-GOLD FROM SELF-FERTILIZED HETEROZYGOTES

The F_2 families derived from crosses between homozygous yellow and old-gold have generally consisted of yellow-flowered and old-gold-flowered plants, in ratios which do not deviate too seriously from the 3 : 1 ratio, when the size of family is taken into account. Twenty-four families of this type are presented in table 3, and it is seen that the totals show 1005 yellow-flowered to 264 old-gold, where 3 : 1 ratios should have given 952 yellow to 317 old-gold. This very material deviation, which is paralleled in some of the other segregations in which the old-gold factor was concerned, might be taken to indicate that, while there is no noticeable inferiority of plants possessing old-gold flowers as compared with those which have yellow-flowers, there is, nevertheless, a probable differential elimination of the old-gold type. There is a special consideration which must be taken into account, however, which may modify the stress to be laid on the deviations shown by the ratios in table 3 and in several of the other tables. The seasons of 1922 and 1923, especially the latter, were very unfavorable because of long periods of drouth, and as a result these years were marked by a failure of many plants, especially of the *Lamarckiana* type, to come to bloom.

In certain families it has been quite obvious that the old-gold type has been slightly later in coming to bloom, than the yellow-flowered type, and it is not unlikely that this slightly delayed development has been effective in decreasing the number of old-gold relatively to the yellow, when conditions were such that only a small portion of the plants secured were induced to bloom. A rather striking illustration of this is seen in families 22155 and 22160, produced by the selfing of two heterozygous sibs in family 21117. In the former, only ten plants bloomed, out of 151, and these were all yellow-flowered, while in 22160 in which 77 plants out of 134 bloomed, there were 61 yellow-flowered to 16 old-gold.

THE YELLOW SEGREGATES FROM SELF-FERTILIZED HETEROZYGOTES

The occurrence of 3 : 1 ratios following self-fertilization of *Oenothera Lamarckiana* having been previously known only in crosses of *Lamarckiana*×*brevistylis*, it seemed important to determine, experimentally, whether the dominant class had the composition which Mendelian theory required, namely, one-third homozygous yellow and two-thirds heterozygous yellow, or whether the 3-to-1 grouping was the result of some other cause or causes than the distribution of a pair of factors in such manner that equal numbers of sperms and equal numbers of eggs carried each member of the pair.

In order to determine this point, the family 2123 (see table 3 and figure 1) was made the subject of an analysis of its dominant type. Of the 35 yellow-flowered plants in this family, 34 were self-fertilized, and the resulting cultures grown in 1923. Unfortunately, there was a seven-weeks drouth in the spring of that year, in the midst of which these plants were transplanted from the greenhouse, where they had received daily irrigation, to the experimental field where the artificial application of water was not feasible. In consequence, while there were very few actual losses by death of the plants, the development was so long retarded that most of the plants resumed the biennial habit which is normal for the species, making large rosettes but developing no stems. Very few of the rosettes lived over the following winter, and no attempt was made to flower any of them in the next season. The scanty data secured from these families are recorded in table 4. With numbers in each family so small, the results are necessarily indecisive, but they are at any rate not in disagreement with the expected results. The 34 families, if distributed as nearly as possible in a 2 : 1 ratio, would have consisted of 23 splitting and 11 non-splitting families, but actually, segregation was manifested in only 13 families and no segregation in 21. When it is observed, however, that one family had no flowering specimen, 3 had only one each, two had only two

TABLE 3

Families from self-fertilized heterozygotes, from crosses between homozygous yellow and vetaurea. See also table 4.

PEDIGREE NUMBERS		SEEDS SOWN	PLANTS SECURED	PERCENTAGE GERMINATION	PLANTS FLOWERED	
Parent	Family				Yellow	Old-gold
198(54)	2047	164	94	57.32	42	20
208(16)	2123	300	62	20.67	35	8
2047(50)	21110	75	12	16.00	3	3
2052(11)	21158	148	22	14.87	11	3
2052(59)	21159	300	129	43.00	88	25
21115(11)	22150	260	160	61.54	65	9
21117(22)	22155	300	158	52.67	10	0
21117(62)	22160	300	134	44.67	61	16
21118(13)	22162	300	40	13.33	16	5
21118(52)	22163	300	173	57.67	85	14
21119(2)	22167	300	103	34.33	35	5
21120(4)	22170	300	73	24.33	21	11
21123(53)	22188	300	149	49.67	45	14
21126(5)	22208	300	90	30.00	50	16
21130(1)	22209	282	135	47.87	7	4
21130(20)	22210	300	144	48.00	71	15
21130(7)	22211	300	76	25.33	5	0
21139(22)	22212	139	45	32.37	10	3
22166(43)	23144	300	160	53.33	100	24
23137(94)	24129	300	45	15.00	21	8
23137(100)	24130	300	132	44.00	94	22
23139(16)	24144	300	96	32.00	49	10
23139(53)	24146	300	58	19.33	31	7
2392(30)	24152	300	111	37.00	60	22
Total					1015	264
Expected (3:1)					*959.25*	*319.75*

each, and so on, and that 24 families had not more than six individuals each, it will be readily seen that a number of potentially splitting families, due alone to the errors of random sampling, will have failed to exhibit a single individual of the recessive type, and such families, consequently, must be grouped with non-splitting families. Assuming that the distri-

TABLE 4

Tests for the occurrence of homozygotes and heterozygotes in Lamarckiana family 2123, which segregated to 35 yellow and 8 vetaurea. All but one of the yellow-flowered plants were self-fertilized.

PEDIGREE NUMBERS		SEEDS SOWN	PLANTS SECURED	PERCENTAGE GERMINATION	PLANTS BLOOMED	
Parent	Family				Yellow	*Vetaurea*
2123(1)	22111	300	84	28.00	56	9
(2)	22112	150	33	22.00	2	–
(3)	22113	300	60	20.00	7	–
(4)	22114	300	85	28.33	16	–
(5)	22115	300	32	10.67	3	2
(6)	22116	300	27	9.00	4	–
(9)	22119	300	24	8.00	10	1
(10)	22120	300	22	7.33	4	1
(11)	22121	300	84	28.00	18	–
(12)	22122	300	83	27.67	6	–
(13)	22123	300	47	15.67	4	–
(14)	22124	300	72	24.00	8	–
(15)	22125	300	77	25.67	2	1
(16)	22126	300	72	24.00	20	1
(17)	22127	300	90	30.00	1	2
(18)	22128	300	115	38.33	4	–
(19)	22129	300	121	40.33	12	2
(23)	22130	300	86	28.67	4	1
(24)	22131	300	48	16 00	5	–
(25)	22132	300	46	15.33	5	–
(26)	22133	300	56	18.67	1	–
(29)	22134	300	65	21.67	–	–
(30)	22135	300	70	23.33	1	–
(31)	22136	188	21	11.17	6	–
(33)	22137	300	57	19.00	4	–
(34)	22138	300	86	28.67	1	1
(35)	22139	300	58	19.33	10	–
(36)	22140	300	53	17.67	4	–
(37)	22141	300	58	19.33	1	–
(38)	22142	300	95	31.67	1	2
(39)	22143	300	88	29.33	5	–
(40)	22144	300	85	28.33	3	3
(42)	22145	300	73	24.33	10	1
(43)	22146	300	55	18.33	2	1

Summary: 20 apparently non-splitting families contain 111 yellow;
14 splitting families contain 129 Y : 28 V, or 4.6 : 1.

bution of the potentially segregating and non-segregating families agrees with the expected 2 : 1 ratio, and calculating the probabilities that the recessive type will be actually included within families having the sizes indicated in table 4 as falling between zero and 6 individuals, inclusively, the theoretical grouping of the 24 families to which these specified limits apply gives 14 apparently non-splitting to 10 splitting. The actual grouping of these 24 families, as seen in the table, is 15 apparently non-splitting and 9 splitting, a very close fit to the theoretical expectation on the assumption that the gametes carrying the factor for old-gold and those carrying its dominant allelomorph are equally numerous in both eggs and sperms. The slight deficiency in the number of splitting progenies is not great enough to be significant in itself, but is in apparent harmony with a significant deficiency in the relative number of recessives as compared with dominants, which resulted from the backcrosses discussed in the next section.

YELLOW-FLOWERED HETEROZYGOTES BACKCROSSED TO OLD-GOLD

The backcrosses of the heterozygotes to the recessive, for the purpose of testing the gametic ratios, are given in table 5. The deviations from the

TABLE 5

Heterozygotes from crosses between yellow and vetaurea, backcrossed to the recessive vetaurea.

PEDIGREE NUMBERS			SEEDS SOWN	PLANTS SECURED	PERCENTAGE GERMINATION	PLANTS FLOWERED	
Parents ♀	♂	Family				Yellow	*Vetaurea*
2047(39)	2047(50)	21112	300	115	38.33	46	44
21115(11)	21108(19)	22151	82	38	46.34	17	5
21117(22)	21108(19)	22157	200	100	50.00	29	39
21117(15)	21108(19)	22158	300	73	24.33	6	5
21117(62)	21108(19)	22159	300	106	35.33	47	28
21118(13)	21108(19)	22164	300	36	12.00	6	5
21119(52)	21108(19)	22165	300	100	33.33	39	15
21119(2)	21108(19)	22168	300	87	29.00	16	9
21123(53)	21108(19)	22189	300	148	49.33	57	44
21126(5)	21108(19)	22200	300	142	47.33	39	27
21139(95)	2223(41)	22213	300	159	53.00	30	2
21139(22)	2223(41)	22214	300	113	37.67	34	20
21139(95)	2223(41)	22215	300	210	70.00	80	42
23139(16)	23144(30)	24145	300	69	23.00	27	22
23139(53)	23144(30)	24147	300	86	28.67	32	31
2392(30)	2391(43)	24153	300	204	68.00	71	58
2392(30)	23144(30)	24154	300	137	45.67	46	38
Total						622	434
Expected (1 : 1)						*528*	*528*

expected 1 : 1 ratio are considerable, but are quite irregular. There is a suggestion in some of the families in this table that there may be present in these particular families a loose linkage of the old-gold factor with a gamete lethal, but this is a subject which will require much more work, and while some experiments are already under way, bearing on this question, they are not yet ready for discussion. It is of special importance to note in this connection, that in most of those families the *vetaurea* grandparents were direct derivatives of *Oenothera Lamarckiana*, in which no gamete lethals have yet been recognized in association with factors of linkage-group I, although the occurrence of abortive pollen and abortive ovules has been repeatedly interpreted as evidence of the occurrence of lethals in this species. At any rate, if the deviations from the 1 : 1 ratio in the families listed in table 5 result from linkage with one or more gamete lethals, it is clear that these lethals are not the same lethals that so strongly dominate the manifestations of factors belonging to group I. The seventeen families listed in table 5 contained a total of 622 yellow-flowered and 434 old-gold,—a deviation from the expected ratio, 528 : 528, too great to refer to errors of sampling, but the nature of the disturbing factors must await the results of further investigation before they can be profitably discussed.

OLD-GOLD COMPLEMENTARY TO SULFUR

Another flower color, also recessive to yellow, has long been known, namely, the *sulfurea* mutation of *Oe. biennis* and of the nearly related species, *Oe. suaveolens*. Sulfur-flowered forms of these two species have been found occasionally in nature in Europe, over a period of time long antedating the beginning of genetical experiments, and they have also arisen by (crossover) mutation in these two species in experimental cultures of STOMPS (1914) and DE VRIES (1918), and once in my own cultures of *Oe. biennis*. The sulfur factor has also been introduced by DAVIS (1916) into *Oe. franciscana* by means of a cross between an *Oe. biennis sulfurea* and *Oe. franciscana*, and I have now, in turn, introduced it into the *Lamarckiana* series of forms. I have made extensive use of the sulfur factor in my genetical experiments, and have reported its close linkage with all of the factors of linkage group I (SHULL 1921a, b, 1923a, b). It has been, until recently, usually associated with one or other of the gamete lethals which control its manifestations in *Oe. biennis* and *Oe. suaveolens*.

One of the first questions which arose, on finding the new recessive flower color, *vetaurea*, was its relationship to the old recessive color, *sulfurea*, and crosses were immediately made to determine this relationship. The ratio of yellow to old-gold in one of the first families containing the latter type, had suggested the possibility that old-gold might be included in the first linkage group, where sulfur had already been demonstrated to lie, and my first thought was that, like several of the eye-color factors of Drosophila, these two color factors were perhaps allelomorphic to each other (SHULL 1921b). This was found, however, to be a wholly erroneous guess, and another quite unexpected relationship between the two colors was discovered, for all the F_1 hybrids between these two types have had the typical yellow flowers characteristic of wild Oenothera species, generally. Crosses between homozygous *vetaurea* and homozygous *sulfurea* are given in table 6, where it is seen that sixteen matings of this type have yielded 2026 yellow-flowered offspring, and none of any other color. I believe this is the first clear case of complementary factors which has been reported in the Oenotheras.

TABLE 6

Families produced by crossing together homozygous vetaurea and homozygous sulfurea. See also table 10.

PEDIGREE NUMBERS			SEEDS SOWN	PLANTS SECURED	PERCENTAGE GERMINATION	PLANTS FLOWERED ALL YELLOW
Parents		Family				
♀	♂					
2047(39)	20250(69)	21120	71	11	15.50	10
2047(39)	20241(101)	21121	300	147	49.00	141
2047(39)	20216(5)	21122	300	139	46.33	134
2047(39)	20258(75)	21124	323	127	39.32	116
20258(111)	2047(39)	21125	300	206	68.67	197
2123(8)	21254(88)	22118	300	183	61.00	91
21225(42)	21113(5)	22149	300	215	71.67	208
22167(41)	22294(49)	2399	300	34	11.33	21
22294(49)	22167(41)	23100	300	192	64.00	186
2390(5)	23141(118)	2482	300	141	47.00	137
23141(118)	2390(5)	2483	300	105	35.00	104
23141(118)	23135(5)	2487	300	148	49.33	145
23141(118)	23144(30)	2488	300	150	50.00	149
23141(118)	23147(92)	24107	300	140	46.67	140
23137(92)	23179(41)	24113	300	116	38.67	64
23141(118)	23137(80)	24123	300	216	72.00	183
Total						2026

FORMULATION OF GENETIC FACTORS FOR FLOWER COLOR

When only two flower colors, yellow and sulfur, were known in the Oenotheras, it was possible to formulate mnemonic symbols for the flower-color factors in two ways, either allowing Y to represent the yellow and *y* the sulfur factor, or letting *S* be the yellow and *s* the sulfur factor. As the former represents the older method, it has commended itself to many geneticists for use except when special considerations render it inappropriate. With the finding of a second factor recessive to yellow, it becomes obvious that the *Yy* formulation is untenable, since *y*, which represents sulfur in one series of crosses, would represent old-gold in another series. Since there is no one factor for yellow, but two or more (possibly many) factors which, acting jointly, produce the yellow flower color, there is no reason for adopting the symbol *Y* as the designation of any one factor. The only alternative, if the conspicuous advantages of mnemonic symbols are to be retained, is to choose the symbol with reference to the recessive trait, as has long been the practice of the students of Drosophila genetics, and of some others, and as has been recommended by the Committee on Genetic Form and Nomenclature, of the AMERICAN SOCIETY OF NATURALISTS (LITTLE 1921). The advantages of choosing symbols on the basis of the recessive traits, from the very start of genetical experiments with any organism, is becoming increasingly obvious, because continued work with the given organism is almost certain to show that what appeared at first to be simple characters, are in fact the resultant of the interactions of two or more genes. Symbols must then be changed to fit the new knowledge, whereas, if the recessive trait is used as the basis of the formulation, the subsequent discovery of new recessive components of a given complex are accommodated by merely adding the new symbols required, without the need of altering the symbolization applied to previously discovered elements of the complex. Adopting, then, the symbols, *s* and *S* for the *sulfurea* factor and its dominant allelomorph, the *vetaurea* factor may be represented by *v* and its dominant allelomorph by *V*. A homozygous yellow will then have the formula (with respect only to the known factors), *SSVV*; homozygous sulfur will be *ssVV*; and homozygous old-gold will be *SSvv*.

THE ORIGIN OF GOLD-CENTER, THE FOURTH FLOWER COLOR IN OENOTHERA

One of the most interesting new problems which presents itself when two factors are found to be complementary to each other, is the character to be expected in the double-recessive (in this case the *vvss*) segregate. In many cases of complementary flower colors the double recessive is phenotypically indistinguishable from one of the singly recessive traits, giving rise to the phenotypic ratio 9 : 3 : 4, and it could not be foreseen in the present case whether the double recessive would be sulfur-colored, old-gold-colored, or of an entirely new color type, and if the last should prove true, the nature of the new color type could not be predicted. Would it be a pure white, as has often proved true in other plants, or would it have some other color?

This interesting question was not decided until late in the summer of 1923, when it was recognized that a plant in family 22197, which had been on a previous occasion recorded as *vetaurea*, was really of a new color type which has since been designated "gold-center" (seg. *aurata*). A search was at once begun for other plants of this new type, and three additional plants of *aurata* were found in family 22197, one in 22171 and two in 22199 (see table 7). Unfortunately, some of the plants in these families had already passed out of bloom, so that it was impossible to check up the classification of all those plants which had been recorded as old-gold or sulfur on previous occasions. It is possible, therefore, that several other *aurata* plants should have been added to those recorded in table 7.

The gold-center flowers have the outer portion of the petals nearly as pale as in sulfur, but of a delicate creamy-white rather than the yellowish-white of sulfurea; the center of the flower is of about the quality of the old-gold flowers (approaching Primuline Yellow) instead of the greenish-yellow center characteristic of sulfur flowers, (see plate 1).

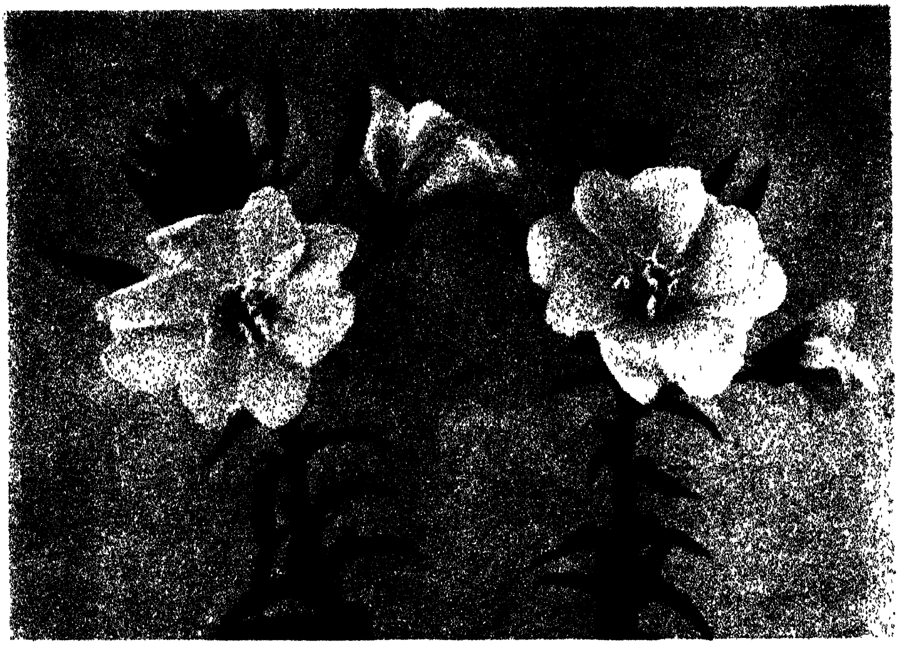
As the flowers fade, it becomes increasingly difficult to distinguish the several color types, but there is never any difficulty in distinguishing them when the flowers are fresh, and experience has shown that fully developed buds give decisive criteria even in the absence of fresh flowers, so that classifications can now be made with full assurance that no unavoidable mistakes are made.

On examining the families included in table 7 it is seen that in only four of them (22171, 22197, 2396 and 2397) is there a close approximation to the typical dihybrid ratio, 9 : 3 : 3 : 1. In all the other families the *sulfurea* type is either completely absent or is present in numbers very much below what would be expected if no gamete lethals had been brought into these crosses from the sulfur-flowered grandparent. In order to properly interpret these segregations, it must be kept in mind that the lethals are linked with the *Ss* factors, which are involved in the yellow and *aurata* phenotypes as well as in *sulfurea*, so that when first-chromosome gamete lethals have accompanied the *s* factor, the ratios will be modified by the complete or partial elimination of both *sulfurea* and *aurata*, and if it has accompanied the *S* factor the percentage of yellow will be reduced.

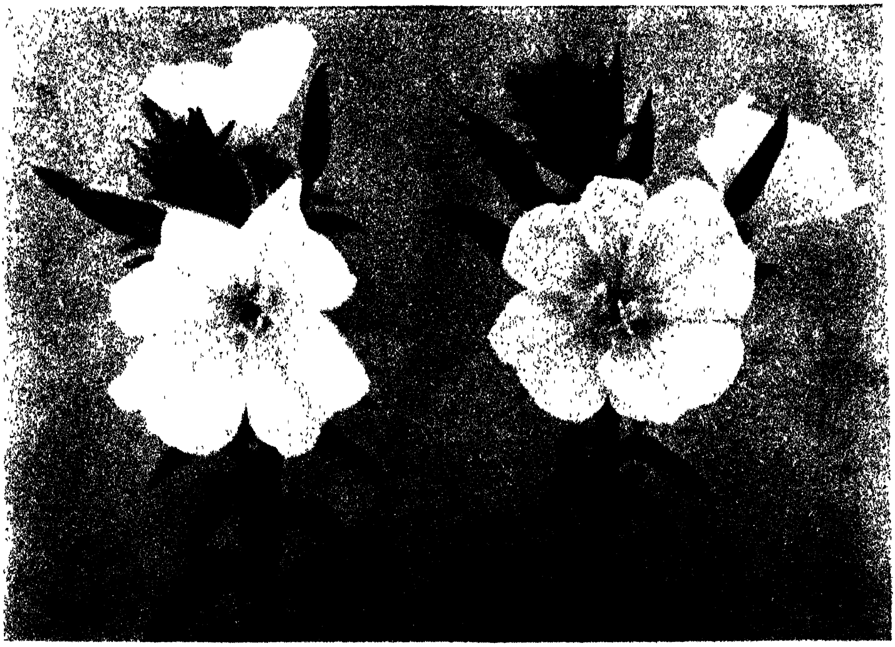
The manner in which these "gamete lethals" produce their characteristic effects is not known, and it may, in fact, turn out that the word "lethal" is in this case a misnomer. This has been recognized from the start and

Legend for Plate 1

The four flower-color types now known in Oenothera. These are, in order, from upper left to lower right, yellow, old-gold, sulfur and gold-center. These figures have been engraved from autochrome photographs, and were all taken from segregates in family No. 24164 which consisted of 57 yellow : 53 old-gold, 48 sulfur : 55 gold-center (see table 16). The engraver has experienced considerable difficulty in reproducing these colors, and it should be noted that the color of the yellow type is distinctly more brilliant than here shown. The correct color being Ridgeway's citron yellow or nearly pale lemon yellow shading to about strontiun yellow at the center. The color of sulfur is approximately correct, being nearly napthaline yellow shaded to citron yellow or strontiun yellow at the center. In old-gold and gold-center, the yellow of the distal portion of the petals is not quite so bright yellow as here shown, being nearer Naples yellow and cream respectively; while the warmer tone toward the center of both of these latter forms approaches primuline yellow being quite noticeably less roseate than here shown. The deviations from the correct color tones are not so great, however, as to entirely destroy the usefulness of this plate.



Yellow Old-gold



Sulfur Gold-center

the word has been used because of its convenience, and always with the mental reservation that the expression is in a sense figurative, and not necessarily accurately descriptive. It has been intended only to symbolize by the expression, "gamete lethals," genetic factors which make it impossible for the egg or sperm which possesses them, to function in fertilization.

If only the yellow and *vetaurea* columns of table 7 are considered, it is seen that there are here also considerable irregularities, some of which are undoubtedly due to inadequate numbers, but for others no satisfactory explanation is yet available. Only further investigations may be competent to explain the considerable deviations from the 3 : 1 ratio in the different families. The totals of these two columns agree closely with the expected 3 : 1 ratio of yellow to *vetaurea*, the summation giving 747 yellow to 229 *vetaurea*, while the theoretical distribution would be 732 yellow to 244 *vetaurea*.

TABLE 7

Families from self-fertilized heterozygotes from crosses between vetaurea and sulfurea.

PEDIGREE NUMBERS		SEEDS SOWN	PLANTS SECURED	PERCENTAGE GERMINATION	PLANTS FLOWERED			
Parent	Family				Yellow *SV*	*Vetaurea Sv*	*Sulfurea sV*	*Aurata sv*
21120(4)	22170	300	73	24.33	21	11	2	–
21120(5)	22171	300	185	61.67	59	20	20	1
21121(24)	22176	300	76	25.33	18	1	–	–
21121(71)	22179	300	120	40.00	23	2	3	–
21122(35)	22182	300	139	46.33	38	1	–	–
21122(96)	22183	300	150	50.00	69	19	3	–
21124(29)	22191	300	106	35.33	40	20	–	–
21124(91)	22192	300	94	31.33	43	1	–	–
21125(21)	22197	300	124	41.33	40	8	5	6
21125(71)	22198	300	210	70.00	116	41	5	–
21125(170)	22199	300	253	84.33	150	60	3	2
22149(5)	2396	300	43	14.33	23	5	5	3
22149(191)	2397	300	184	61.33	80	35	20	5
23143(27)	24191	300	84	28.00	27	5	–	–

Two of the families (2396 and 2397) which gave approximately the typical dihybrid ratio, 9 : 3 : 3 : 1, are of special interest because they were derived from a cross between a strain of *Oe. franciscana sulfurea* which was thought to possess no gamete lethals and *Oe. decipiens vetaurea* which is believed to contain neither of the zygote lethals of *Oe. Lamarckiana* from which it has been indirectly derived. The near agreement of these ratios with those of typical Mendelian hybrids tends to confirm the assumptions made regarding the absence of lethals from the two P_1 plants 21225(42) and 22113(5). A more comprehensive study of this question will be presented in a later section (page 225). Whether lethals were present in the other crosses in this table had not been predictable.

BACKCROSSES OF DIHETEROZYGOUS YELLOW, *SsVv*, TO OLD-GOLD

The discovery of the compound nature of the yellow flower color has opened up a number of new problems which will be considered in the following sections. For instance, it is important to know whether the synthesized yellow has any peculiarities of genetical behavior, as compared with the homozygous yellow of the wild type. Backcrosses to the several recessive types give the best means of testing this question. In table 8 are given the results of crossing nine diheterozygous yellow-flowered plants with a pure *Lamarckiana vetaurea* pollinator. In terms of the formulation here adopted these crosses represent the combination *SsVv*×*SSvv*, and should theoretically yield 1 : 1 ratios of yellow (*SSVv*+*SsVv*) to *vetaurea* (*SSvv*+*Ssvv*). It is seen that, on the average, this expectation is closely approximated, for the totals of the nine matings consist of 337 yellow to 363 *vetaurea*, as compared with 350 of each type theoretically expected. When attention is given, however, to the individual families, it is seen at once that there are, in a number of these families, too great discrepancies between the observed and the expected ratios, to be explained by errors of random sampling. What the nature of the disturbing factors may be is not yet known.

The two *sulfurea* individuals in family 22194 may be plausibly attributed to an accident of technique which resulted in a self-fertilization of the diheterozygous mother, 21124(91).

BACKCROSSES OF DIHETEROZYGOUS YELLOW, *SsVv*, TO SULFUR

The corresponding backcrosses of the F_1 hybrids between sulfur and old-gold, to *sulfurea*, are given in table 9. These crosses may be expressed by the formula *SsVv*×*ssVV*. The expectation parallels that of the preceding section, except that the 50 percent non-yellow are, in the present case, sulfur instead of old-gold. The yellow segregates should be genotypically of two sorts (*SsVV* and *SsVv*), and the *sulfurea* segregates likewise of two genotypes (*ssVV* and *ssVv*). If we separate out three of the eleven families in table 8 (22174, 22196 and 22202) which show rather conspicuous evidence of the effects of gamete lethals, most of the remaining families deviate but little from the expected 1 : 1 ratios, but the totals for these eight families, 496 yellow and 421 sulfur, still deviate too much from the 458.5 of each required by the 1:1 ratio.

TABLE 8

The yellow-flowered F_1 plants from crosses of velaurea and sulfurea, backcrossed to velaurea.

PEDIGREE NUMBERS			TYPE OF CROSS	SEEDS SOWN	PLANTS SECURED	PERCENTAGE GERMINATION	PLANTS FLOWERED		
Parents		Family					Yellow	*Velaurea*	*Sulfurea*
♀	♂								
21120(4)	21108(19)	22172	(V×S)×V	190	74	38.95	5	2	-
21120(5)	21108(19)	22173	(V×S)×V	300	99	33.00	24	13	-
21171(71)	21108(19)	22180	(V×S)×V	300	131	43.67	43	9	-
21122(96)	21108(19)	22185	(V×S)×V	300	92	30.67	30	34	-
21124(91)	21108(19)	22194	(V×S)×V	300	210	70.00	40	32	2*
21124(29)	21108(19)	22195	(V×S)×V	150	48	32.00	3	6	-
21125(21)	21108(19)	22203	(S×V)×V	300	192	64.00	48	70	-
21125(71)	21108(19)	22204	(S×V)×V	300	216	72.00	66	90	-
21125(170)	21108(19)	22206	(S×V)×V	300	241	83.33	78	107	-
Totals							337	363	2*
Expected							*350*	*350*	-

* Probably the result of an accidental self-fertilization.

TABLE 9

The yellow-flowered F_1 plants from crosses between vetaurea and sulfurea, backcrossed to sulfurea. See also table 10.

PEDIGREE NUMBERS			TYPE OF CROSS	SEEDS SOWN	PLANTS SECURED	PERCENTAGE GERMINATION	PLANTS FLOWERED	
Parents ♀	Parents ♂	Family					Yellow	*Sulfurea*
21120(4)	21225(27)	22174	(V×S)×S	300	71	23.67	29	1
21120(5)	21225(27)	22175	(V×S)×S	300	107	35.67	31	17
21121(24)	21212(39)	22177	(V×S)×S	250	84	33.60	16	29
21121(71)	21212(39)	22181	(V×S)×S	300	105	35.00	33	27
21122(35)	21195(31)	22186	(V×S)×S	300	109	36.33	29	11
21122(96)	21195(31)	22187	(V×S)×S	300	191	63.67	58	56
21124(91)	21225(27)	22193	(V×S)×S	300	163	54.33	66	50
21124(29)	21225(27)	22196	(V×S)×S	300	77	25.67	38	2
21125(21)	21225(27)	22202	(S×V)×S	300	191	63.67	9	96
21125(71)	21225(27)	22205	(S×V)×S	300	282	94.00	146	127
21125(170)	21225(27)	22207	(S×V)×S	300	243	81.00	117	104
Totals							572	520
Expected (1 : 1)							*546*	*546*

TESTS OF OLD-GOLD- AND SULFUR-FLOWERED SEGREGATES IN F_2 FAMILIES FROM CROSSES BETWEEN OLD-GOLD AND SULFUR

An interesting corollary of the Mendelian interpretation of the distribution of the flower-color factors here under consideration, is the inference that, whereas the sulfur and old-gold types were known heretofore only in the homozygous state, *ssVV* and *SSvv*, respectively, the segregates having these two phenotypic characters should be divisible in each case into homozygous and heterozygous classes with respect to their genotypic constitutions, the sulfur-flowered plants being of the two genotypes, *ssVV* and *ssVv*, and the old-gold of the two genotypes *SSvv* and *Ssvv*.

In order to discover whether these new heterozygous *sulfurea* and *vetaurea* classes were actually present among the segregates from the crosses between old-gold and sulfur, eight *vetaurea* segregates in families 22198 and 22199 were self-fertilized and also crossed with pollen from an *Oe. franciscana sulfurea* (22294(79)), and six *sulfurea* segregates in families 22194, 22198 and 22199 were self-fertilized and at the same time crossed with pollen from an *Oe. Lamarckiana vetaurea* (2220(40)). The results of these fourteen double tests are assembled in table 10, grouped according to the demonstrated genotypes. Of the 8 *vetaurea* segregates tested, 3 proved to be homozygous (*SSvv*) yielding only *vetaurea* when selfed (the unexpected presence of 13 yellows in family 23111 being certainly due to some technical error), and when crossed with homozygous sulfur produced only yellow-flowered offspring.

The remaining 5 *vetaurea* plants tested were found to be heterozygous (*Ssvv*), splitting to *vetaurea* and *aurata* when selfed and giving yellow and *sulfurea* in approximately equal numbers when crossed with homozygous sulfur. In all of the selfings of this group of plants there was a very marked deficiency in the number of *aurata* plants, the totals showing 692 *vetaurea* to 13 *aurata*. This is interpreted as meaning that the *sulfurea* factor (*s*) in all of these five plants was associated with a sperm lethal. That there was no egg lethal present is shown by the fact that the crosses to sulfur gave a total of 437 yellow to 445 sulfur, a very close fit to the expected 1 : 1 ratio.

Of the five sulfur-flowered segregates tested, two proved to be homozygous (*ssVV*) (third section of table 10), giving only sulfur when selfed and only yellow when crossed with *vetaurea*, while four (fourth section of table 10) were found to be heterozygous (*ssVv*), giving both sulfur and aurata when selfed, and both yellow and *vetaurea* when backcrossed to *vetaurea*.

The self-fertilizations totalled 422 *sulfurea* : 94 *aurata*, or 4.5 : 1, which bears the same relation to 3 : 1 that is often seen in other cases, in which an adequate explanation is found in the assumption that the recessive is slightly less effective, physiologically, than the dominant type. The backcrosses of these same plants, however, show such an excess of *vetaurea* over yellow in all families except that derived from 22194(53), that we must assume that the inadequacy of the sulfur type is a gametic rather than a somatic one, or possibly that it is both gametic and somatic.

Notwithstanding such important discrepancies as these, it is seen that this series of tests demonstrates the occurrence of heterozygous as well as homozygous *vetaurea* and *sulfurea* individuals in the progenies of the diheterozygous yellows, and the numbers of these several types,—3 *SSvv* : 5 *Ssvv*, and 2 *ssVV* : 4 *ssVv*,—are as close as possible to the 1 : 2 ratios expected.

GOLD-CENTER BREEDS TRUE

The finding of the new flower color, "gold-center," (seg. *aurata*) gave the basis of a new set of analyses of the flower color characters. These have not yet been carried out so extensively as have the breedings with old-gold, but they are, nevertheless, sufficiently consistent that there can be no doubt as to the validity of the conclusions to which they lead. It was assumed, at once, on the discovery of the gold-center plants, that they were the double-recessive segregates (*ssvv*) from the cross between sulfur and old-gold, and the correctness of this assumption has been demonstrated by the series of tests discussed in the last section and recorded in table 10. It was also recognized, however, that no other new character which originated as a definite segregate from a previous cross has been reported in the Oenotheras, and it has seemed desirable, therefore, to submit the new type to a full series of breeding tests in combination with the other color types. In table 11 it is seen that four families produced by self-fertilization of plants of the *aurata* or gold-center type yielded 489 *aurata* offspring, and none of any other color. This result agrees with what is expected of recessive types, generally, on being self-fertilized.

RECESSIVENESS OF GOLD-CENTER TO ALL OTHER COLORS

When gold-center is crossed with homozygous strains of yellow, sulfur or old-gold, it is found to be in all cases completely recessive. Table 12 gives the results of five crosses with homozygous yellow, which yielded a total of 805 yellow-flowered progeny and 1 *aurata*, the last being un-

TABLE 10

Self-fertilizations of segregates in F_2 families from crosses between velaurea and sulfurea, and backcrosses of the same plants to sulfurea and velaurea, respectively, to discover the several genotypes present. Arranged in four sections according to the demonstrated genotype.

PEDIGREE NUMBERS			TYPE OF CROSS	SEEDS SOWN	PLANTS SECURED	PERCENTAGE GERMINATION	PLANTS FLOWERED			
Parents ♀	Parents ♂	Family					Yellow	*Velaurea*	*Sulfurea*	*Aurata*
22198(15)	22198(15)	23111	V×self	300	138	46.00	13*	94	-	-
22198(15)	22294(79)	23112	V×S	300	235	78.33	205	-	-	-
22199(197)	22199(197)	23127	V×self	300	234	78.00	-	226	-	-
22199(197)	22294(79)	23128	V×S	300	157	52.33	146	-	-	-
22199(238)	22199(238)	23129	V×self	300	5	1.67	-	5	-	-
22199(238)	22294(79)	23130	V×S	300	156	52.00	150	-	-	-
Totals (3 individuals)		V×self (omitting 13 yellow in 23111)					-	325	-	-
		V×S					501	-	-	-
Demonstrated genotype, *SSvv*										
22198(38)	22198(38)	23113	V×self	300	202	67.33	-	169	-	2
22198(38)	22294(79)	24114	V×S	300	254	84.67	112	-	130	-
22198(43)	22198(43)	23115	V×self	300	75	25.00	-	62	-	-
22198(43)	22294(79)	23116	V×S	300	176	58.67	75	-	83	-
22198(55)	22198(55)	23117	V×self	300	146	48.67	-	108	-	1
22198(55)	22294(79)	23118	V×S	300	171	57.00	75	-	89	-
22199(96)	22199(96)	23123	V×self	300	244	81.33	-	186		5
22199(96)	22294(79)	23124	V×S	300	201	67.00	112	-	75	-
22199(162)	22199(162)	23125	V×self	300	198	66.00	-	167	-	5
22199(162)	22294(79)	23126	V×S	300	135	45.00	63	-	68	-
Totals (5 individuals)		V×self					-	692	-	13
		V×S					437	-	445	-
Demonstrated genotype, *Ssvv*										
22194(8)	22194(8)	23101	S×self	300	264	88.00	-	-	127	-
22194(8)	2220(40)	23102	S×V	300	151	50.33	110	-	-	-
22199(16)	22199(16)	23131	S×self	300	95	31.67	-	-	90	-
22199(16)	2220(40)	23132	S×V	300	167	55.67	151	-	-	-
Totals (2 individuals)		S×self					-	-	217	-
		S×V					261	-	-	-
Demonstrated genotype, *ssVV*										
22194(53)	22194(53)	23103	S×self	300	190	63.33	-	-	53	8
22194(53)	2220(40)	23104	S×V	300	92	30.67	29	14	-	-
22198(20)	22198(20)	23119	S×self	300	222	74.00	-	-	128	30
22198(20)	2220(40)	23143	S×V	300	252	84.00	89	122	-	-
22198(175)	22198(175)	23120	S×self	300	232	77.33	-	-	152	25
22198(175)	2220(40)	23121	S×V	300	230	76.67	74	104	-	-
22199(91)	22199(91)	23133	S×self	300	134	44.67	-	-	89	31
22199(91)	2220(40)	23134	S×V	300	91	30.33	31	54	-	-
Totals (4 individuals)		S×self					-	-	422	94
		S×V					223	294	-	-
Demonstrated genotype, *ssVv*										

*Undoubtedly due to some technical error.

TABLE 11

Self-fertilizations of the new flower color, gold-center (aurata).

PEDIGREE NUMBERS			SEEDS SOWN	PLANTS SECURED	PERCENTAGE GERMINATION	PLANTS FLOWERED, ALL *aurata*
Parents		Family				
♀	♂					
22197(94)	22197(94)	23105	300	175	58.33	139
22199(33)	22199(33)	23122	300	115	38.33	93
2396(2)	2396(2)	24155	300	176	58.67	137
23133(59)	23133(59)	24187	300	166	55.33	120
Total						489

TABLE 12

Crosses of aurata to homozygous yellow.

PEDIGREE NUMBERS			SEEDS SOWN	PLANTS SECURED	PERCENTAGE GERMINATION	PLANTS FLOWERED	
Parents		Family				Yellow	*Aurata*
♀	♂						
22197(94)	22293(76)	23107	300	65	21.67	63	–
22197(94)	2295(43)	23108	300	269	89 67	232	–
22197(94)	22166(7)	23109	300	76	25 33	70	–
22197(94)	22218(34)	23110	300	230	76.67	219	1*
22218(34)	22197(94)	23146	296	235	79.39	221	–
Total						805	1*

* Undoubtedly the result of an accidental self-pollination.

TABLE 13

Crosses of aurata to homozygous vetaurea.

PEDIGREE NUMBERS			SEEDS SOWN	PLANTS SECURED	PERCENTAGE GERMINATION	PLANTS FLOWERED ALL *vetaurea*
Parents		Family				
♀	♂					
23137(92)	23133(59)	24108	300	153	51 00	86
23133(59)	23137(92)	24109	300	198	66 00	174
23133(59)	23144(30)	24142	300	205	68.33	199
23133(59)	23135(5)	24188	300	194	64 67	178
Total						637

doubtedly the result of an accidental self-fertilization of the *aurata* mother, 22197(94). Table 13 contains the data from four crosses of *aurata* with homozygous *vetaurea*, the total progeny consisting of 637 *vetaurea* and none of any other color. Table 14 gives the corresponding results of three crosses between *aurata* and homozygous *sulfurea*, the total offspring of which consisted of 620 *sulfurea*, and none of any other color.

This last case is particularly interesting from the fact that gold-center was originally recorded as an old-gold, and it is in danger of being occasionally confused with old-gold unless the classification is made from fresh flowers or from mature buds. Had old-gold instead of gold-center been used in the three crosses of table 14, the progenies would have consisted solely of yellow-flowered plants instead of sulfur-flowered, so that the cross to *sulfurea* may be used as a crucial test in any case in which doubt is entertained as to whether a given plant is old-gold or gold-center. As already stated, adequate experience in separating the color types renders the use of this crucial test unnecessary.

TABLE 14

Crosses of aurata to homozygous sulfurea.

PEDIGREE NUMBERS			SEEDS SOWN	PLANTS SECURED	PERCENTAGE GERMINATION	PLANTS FLOWERED ALL *sulfurea*
Parents ♀	Parents ♂	Family				
22197(94)	22294(49)	23106	300	249	83 00	227
23141(118)	23133(59)	2491	300	264	88 00	245
23133(59)	23179(41)	24143	300	179	59 67	148
Total						620

SEGREGATIONS OF GOLD-CENTER FROM ITS CROSSES WITH SULFUR

In table 15 is given the result of one self-fertilization, and five back-crosses of heterozygous sulfur, *ssVv*, to gold-center, *ssvv*. In only one case (family 24184) is there a close agreement with the expected ratio. In

TABLE 15

Segregation of aurata from crosses with sulfurea.

PEDIGREE NUMBERS			SEEDS SOWN	PLANTS SECURED	PERCENTAGE GERMINATION	PLANTS FLOWERED	
Parents ♀	Parents ♂	Family				*Sulfurea*	*Aurata*
23106(16)	23106(16)	24172	300	165	55 00	90	15
Expected (3:1)						*78.75*	*26.25*
23105(125)	23106(16)	24173	300	155	51.67	73	38
23106(16)	23133(59)	24174	300	130	43.33	54	32
23114(125)	23113(80)	24184	300	182	60.67	78	79
23114(125)	23113(179)	24185	300	133	44.33	62	48
23114(125)	23113(192)	24186	300	182	60.67	96	51
Total						363	248
Expected (1 : 1)						*305.5*	*305.5*

all other cases there is a deficiency of the gold-center class. The progeny from self-fertilization shows a deviation of 11.25 ± 2.99, and the totals from the backcrosses give a deviation of 57.5 ± 8.34. These deviations are probably not due to lethal factors, but perhaps to some slight inferiority of the recessive type, as is so often indicated in other organisms.

THE DOUBLE-DOMINANT SEGREGATES IN DIHYBRID F_2 FAMILIES

We have seen in table 7 that families 2396 and 2397 yielded a close approximation to the 9 : 3 : 3 : 1 ratio. These two families were especially interesting because they represented a cross between *decipiens vetaurea*, believed to be free from zygote lethals, and a strain of *franciscana sulfurea*, which was assumed to have lost the gamete lethals with which the *sulfurea* factor has been usually associated. The presentation of nearly typical dihybrid ratios in the second generation of this cross seemed to confirm the assumptions regarding the freedom of the two P_1 plants from lethals. As no one else has ever reported typical dihybrid ratios in Oenothera, it was thought desirable to determine experimentally whether the 9 : 3 : 3 : 1 ratio in these families has the significance that it has in typical Mendelian breeding. For this purpose an analysis of the genotypes represented in the double-dominant phenotypic class, yellow, was undertaken by means of backcrosses to the double-recessive type, gold-center. The nine individuals of the double-dominant class in the theoretical 9 : 3 : 3 : 1 ratio, consist of 1 pure double-dominant (*SSVV*), 2 monoheterozygotes with respect to the first factor (*SsVV*), 2 monoheterozygotes with respect to the second factor (*SSVv*), and 4 diheterozygotes (*SsVv*). Backcrosses of plants having these several genotypic constitutions, to the double recessive (*ssvv*), should give four kinds of families in the ratio 1 : 2 : 2 : 4.

Five yellow segregates in family 2396 and nine in family 2397 were used to pollinate the flowers on sixteen different branches of a single large *aurata* segregate, 2396(2), and a seventeenth branch of the same plant was self-fertilized (see table 11). The sixteen families derived from these backcrosses were grown during the past season (1925) and the results are given in table 16. The four sections of the table correspond with the four genotypes expected on a Mendelian basis, and the fit to the theoretical ratio, 1 : 2 : 2 : 4, is as close as possible,—much closer than could reasonably be expected in a test of only 16 individuals.

When consideration is given to the ratios in the individual families, however, irregularities are seen, very much too great to be attributed to errors of sampling, though the theoretical ratios are closely approached in a number of cases. Thus, all of the ratios of the third section (*SsVV*) agree very well indeed with the expected 1 : 1 ratio, the deviation being 11.5 and the probable error, ±8.27. The last ratio of section 2 is sufficiently near to the 1 : 1 ratio, and at least one family (24164) in the last section shows a very close approach to the 1 : 1 : 1 : 1 ratio, a gametic ratio that, so far as I know, has never before been reported in the Oenotheras. The irregularities in the other families cannot at present be accounted for, and must await the light of a fuller analytical test. The most aberrant family (24162) is of special interest because of the suggested presence of a gamete lethal cutting down the *sulfurea* and *aurata* classes. Two of the families (24163 and 24168) in the second section also suggest a rather loose linkage between a gamete lethal and the old-gold factor, reducing the old-gold class to about half the expected size. An important point to be made, however, is, that if the mentioned discrepancies are in fact the result of lethal factors, there are no indications that the lethals which affect the *vetaurea* class are the same as those which modify the *sulfurea* class. All the evidence indicates that *vetaurea* and *sulfurea* belong to different linkage groups.

INDEPENDENCE OF THE OLD-GOLD FACTOR FROM OTHER FACTORS OF THE FIRST LINKAGE GROUP

While no extensive series of tests have been made to determine the linkage or independence of the old-gold factor, *v*, and its allelomorph *V*, in relation to other factors of linkage-group I, a number of the families listed in the foregoing tables have involved other first-chromosome factors besides the sulfur factor which was specifically under consideration. For the sake of simplicity no attention has been given to these other factors in the present discussion. It is sufficient for the present to simply cite an example or two to indicate the relation of *vetaurea* to other first-chromosome factors, and to summarize the experiences to date by the statement that thus far no situation has presented itself which would suggest a linkage between old-gold and any factor included in the first linkage group.

In 1921 a cross was made between one of the original *vetaurea* plants, 2047(39), which had the form of *Lamarckiana*, tall with green-hypanthium buds, and a *rubricalyx nanella*, 20202(17), dwarf, with red hypanthia. The F_1 family (21126) consisted of 20 tall yellow-flowered plants with *rubricalyx* buds (see table 2). One of the plants of this family 21126(5), self-fertilized, produced the F_2 family 22208 listed in table 3. The tall *versus* dwarf stems and red *versus* green hypanthia are closely linked

TABLE 16

Double-dominant yellow-flowered plants in (sulfurea×velaurea)F_2, backcrossed to the double-recessive aurata.

PEDIGREE NUMBERS			SEEDS SOWN	PLANTS SECURED	PERCENTAGE GERMINATION	FLOWER COLORS OF OFFSPRING				GENOTYPE OF YELLOW-FLOWERED PARENT PLANTS
Parents ♀	♂	Family				Yellow	*Velaurea*	*Sulfurea*	*Aurata*	
2396(2)	2396(12)	24158	300	196	65.33	159	-	-	-	*SSVV* (1 plant)
2396(2)	2397(8)	24163	300	140	46.67	75	43	-	-	
2396(2)	2397(57)	24168	300	173	57.67	90	44	-	-	*SSVv* (3 plants)
2396(2)	2397(62)	24171	300	219	73.00	97	83	-	-	
Total						262	170			
Expected (1 : 1)						*216*	*216*			
2396(2)	2396(13)	24159	300	211	70.33	86	-	90	-	
2396(2)	2396(40)	24161	300	140	46 67	34	-	44	-	*SsVV*
2396(2)	2397(50)	24165	300	249	83.00	103	-	105	-	(4 plants)
2396(2)	2397(60)	24169	300	177	59.00	66	-	73	-	
Total						289		312		
Expected (1 : 1)						*300.5*		*300.5*		
2396(2)	2396(1)	24156	300	144	48.00	22	20	23	14	
2396(2)	2396(7)	24157	300	255	85.00	46	40	54	61	
2396(2)	2396(15)	24160	300	194	64.67	39	35	49	20	
2396(2)	2396(41)	24162	300	153	51.00	70	26	4	3	*SsVv*
2396(2)	2397(48)	24164	300	228	76.00	57	53	48	55	(8 plants)
2396(2)	2397(51)	24166	300	173	57.67	46	28	30	12	
2396(2)	2397(54)	24167	300	216	72.00	43	32	54	27	
2396(2)	2397(61)	24170	291	198	68.04	38	34	54	30	
Total						361	268	316	223	
Expected (1 : 1 : 1 : 1)						*292*	*292*	*292*	*292*	
Difference						+69	−24	+24	−69	

together in the first chromosome, and as the two dominant factors, tall stems and *rubricalyx* buds, were brought into the cross from opposite sides, they segregated in the form of a typical repulsion-phase ratio, namely 1 tall with green hypanthium : 2 tall with red hypanthium : 1 dwarf with red hypanthium, the actual numbers being 14 : 39 : 13. The distribution of the flower colors was as follows:

	Tall green	*Tall red*	*Dwarf red*
Yellow	10	29	11
Old-gold	4	10	2

The close parallel in the two color classes is obvious. Putting all together in the form of a single ratio gives the numbers:

Observed	10 : 29 : 11 : 4 : 10 : 2
Expected (3 : 6 : 3 : 1 : 2 : 1)	*12* : *25* : *12* : *4* : *8* : *4*

The fit is remarkably close, in view of the rather small number of individuals available and the six-term phenotypic ratio.

The independence of old-gold from *rubricalyx* bud color was also shown by a cross of the same individual (21126(5)) whose self-fertilized progeny has just been considered, to a *Lamarckiana vetaurea*, 21108(19), this constituting a backcross of a plant heterozygous for yellow flowers and *rubricalyx* buds, to the double recessive with old-gold flowers and green hypanthia. The resulting family (22200) consisted of 15 *rubricalyx* yellow, 24 green-hypanthium yellow, 14 *rubricalyx vetaurea* and 13 green-hypanthium *vetaurea*, the 1 : 1 : 1 : 1 ratio expected on the basis of independence requiring 16.5 plants in each class. The value of χ^2 is 4.67 and P = 0.201.

THE INDEPENDENCE OF OLD-GOLD AND *brevistylis*

Having now shown that the *vetaurea* factor is independent of *sulfurea*, *nanella*, *rubricalyx* and the first-chromosome lethals, there remains the question as to its relation to the second-chromosome factor for short styles, *brevistylis*.

Self-fertilizations and backcrosses of plants in which both the *vetaurea* factor v and the *brevistylis* factor b_r were heterozygous have given a decisive answer to this question, as will be obvious from tables 17 and 18. The very close agreement with the 3 : 1 ratio in every color class in table 17 and with the 1 : 1 : 1 : 1 ratio in table 18, tells impressively the story of independence. The greatest deviation occurs in the *vetaurea* class in table 17 where it is 6.5 and the probable error ±2.89. It will be noted that the backcrosses entered in table 18 involve the same three heterozygotes whose self-fertilization progenies are given in the last three lines of table 17. The most interesting of these families is 24139, whose parent was heterozygous not only for *velaurea* and *brevistylis* but also for *sulfurea.* Had no "pollen lethal" been associated with the sulfur factor in linkage-group I, the sulfur-flowered segregates should have been as numerous as the old-gold segregates and there should have been also a group of *aurata* offspring as well. That the parent of this family was producing *S*- and *s*-bearing eggs in equal numbers, in agreement with Mendelian theory, and that the sulfur factor is likewise independent of *brevistylis* was shown by a backcross of 23109(23) to the double recessive *sulfurea brevistylis,* 23179(41). The resulting family (24141) consisted of:

	Yellow		*Sulfur*		*Unclassified*	
	Long styled	*Brevistylis*	Long-styled	*Brevistylis*	Long-styled	*Brevistylis*
Observed	59	61	42	38	6	5
Expected	*50*	*50*	*50*	*50*	-	-
Difference	+ 9	+11	− 8	− 12	-	-

The value of χ^2 is in this case 8.2, giving P = .0427. This low value of P shows that the deviation is almost certainly in part due to other causes than the errors of random sampling, but the ratio between long-styled and *brevistylis* is almost exactly 1 : 1 in each color class, and this is the criterion of independence between the color factors and the style-length factors, in which we are here specifically interested. If the ratio 59 : 61: 42 : 38 be considered as a linkage ratio, it is seen that the crossover classes are actually slightly, though insignificantly, in excess of the non-crossover classes.

DISCUSSION

The data presented in the foregoing sections of this paper make it clear that the new flower-color factor, "old-gold," is distributed in heredity more nearly in accord with the typical Mendelian model, than any other Oenothera factor which has yet been studied, with exception of the *brevistylis* factor, which latter has always heretofore been the outstanding exception to the seeming perversities of the genetical situation in the Oenotheras.

That there are very serious deviations from the "expected" ratios in individual families must be conceded, and there is no inclination to minimize the importance of these discrepancies when I point out that the remarkable agreement with all the larger implications of the Mendelian scheme of distribution indicates that, compared with these fundamenta

TABLE 17

Segregations following self-fertilization in crosses involving vetaurea and brevistylis.

PEDIGREE NUMBERS		TYPE OF CROSS	SEEDS SOWN	PLANTS SECURED	PERCENTAGE GERMINATION	YELLOW		*Vetaurea*		*Sulfurea*		UNCLASSIFIED	
Parent	Family					Long-styled	*Brevistylis*	Long-styled	*Brevistylis*	Long-styled	*Brevistylis*	Long-styled	*Brevistylis*
21118(13)	22162	$(VL\times Ybr)F_1$	300	40	13.33	14	2	3	2	–	–	–	–
21118(52)	22163	$(VL\times Ybr)F_1$	300	173	57.67	66	19	12	2	–	–	2	–
22166(43)	23144	$(VL\times Ybr)F_2$	300	160	53.33	78	22	19	5	–	–	6	2
23109(23)	24139	$(AL\times Ybr)F_1$	300	210	70.00	85	38	32	6	9	2	3	2
23139(16)	24144	$(YL\times Vbr)F_1$	300	96	32.00	38	11	7	3	–	–	–	–
23139(53)	24146	$(YL\times Vbr)F_1$	300	58	19.33	21	9	7	0	–	–	–	–
Totals						302	101	80	18	9	2	11	4
Expected (3 : 1)						*302.25*	*100.75*	*73.5*	*24.5*	*8.25*	*2.75*	*11.25*	*3.75*

TABLE 18

Backcrosses of the diheterozygote to the double recessive in crosses involving vetaurea and brevistylis.

PEDIGREE NUMBERS			TYPE OF CROSS	SEEDS SOWN	PLANTS SECURED	PERCENTAGE GERMINATION	YELLOW		*Vetaurea*		UNCLASSIFIED	
Parents ♀	Parents ♂	Family					Long-styled	*Brevistylis*	Long-styled	*Brevistylis*	Long-styled	*Brevistylis*
23109(23)	23144(30)	24140	$(AL\times Ybr)F_1\times Vbr$	100	10	10.00	2	2	1	2	1	–
23139(16)	23144(30)	23145	$(YL\times Vbr)F_1\times Vbr$	300	69	23.00	18	11	9	13	1	1
23139(53)	23144(30)	24147	$(YL\times Vbr)F_1\times Vbr$	300	86	28.66	15	16	18	13	2	–
Total							35	29	28	28	4	1
Expected (1 : 1 : 1 : 1)							*30*	*30*	*30*	*30*	–	–

agreements, the deviations of ratios in single families are of minor significance. Further intensive and extensive analyses may be expected in time to differentiate between the genetic, physiological and environmental causes of these irregularities in the ratios.

The demonstration that the old-gold factor is independent of the sulfur factor and other factors linked with sulfur in linkage-group I, and that it is also independent of *brevistylis*, the "marker" of linkage-group II, indicates that it is the first-discovered factor of linkage-group III, for which it may well serve as the "marker." That it is proper to speak of these as *linkage groups*, and not merely as independent factors, has been shown by the discovery of a second factor closely linked with old-gold in group III (SHULL 1925), and I have evidence that there is a second factor in group II, linked with *brevistylis*.

The chief importance of these new discoveries in Oenothera, as in purely scientific genetical experiments, generally, is in the bearing they have as indicators of the nature of the mechanism which controls the distribution of the hereditary factors. It has been demonstrated by the work of many investigators, and by evidence from several different sides, that independent inheritance (Mendelian) results from the inclusion of the genes in different pairs of chromosomes. Correlatively, the work especially of MORGAN and his students and collaborators has proven that the linkage of factors in Drosophila depends upon the inclusion of the linked factors in a single chromosome pair, and this interpretation has been accepted for many other organisms in which linked genes have been studied by other investigators. I have accepted the probable correctness of this same interpretation as applied to the linkage phenomena in Oenothera.

GATES (1908) and DAVIS (1910) have found a telosynaptic arrangement of the chromosomes in Oenothera, and GATES (1923) holds that this fact is an obstacle to the occurrence of partial linkage in this genus. The genetical evidence of linkage with crossing over is unequivocal, however, and every season adds new and striking examples of it in my cultures.

CLELAND'S (1922, 1923, 1924, 1925, 1926) brilliant work on the chromosome groupings in Oenothera has brought to light a behavior among the chromosomes, which at once suggests the possibility that the linkages in Oenothera might be due to the cohesion of non-homologous chromosomes, and their separation during meiosis in such manner that the maternal members of the group are drawn to one pole and the paternal members to the other pole of the spindle. CLELAND finds a marked tendency for the alternate members of the circles of cohering chromosomes to go to the same pole. If this behavior is of general occurrence it need only be assumed that the paternal and maternal chromosomes alternate with each other in the circles, to account for the formation of gametes predominantly of two kinds, the one possessing the entire maternal complex in so far as that complex is included in the chromosomes of the circle, and the other possessing the corresponding paternal complex. Crossing over would result, then, simply through an irregularity which would reverse the usual program with respect to one or more of the pairs of chromosomes included in the circle, so that one or more maternal chromosomes would migrate to the otherwise paternal pole and *vice versa*.

Cleland has reported an amount of irregularity in the separation of the chromosomes that would apparently account for a very much greater degree of genetic variability than has been found by students of Oenothera genetics, and this is one of the difficulties, perhaps, in identifying chromosomal cohesion as the mechanism of linkage in this group.

There are several other difficulties which seem to me to stand in the way of accepting the hypothesis that the linkage phenomena in Oenothera result from the end-to-end cohesion of non-homologous chromosomes. One of these I have already pointed out, namely, that in the first linkage group there are now known about twice as many factors as there are haploid chromosomes in Oenothera. It might seem necessary on this account to assume the inclusion of more than one of these factors in a single chromosome, but this is not yet a *necessary* conclusion, since all of the known differential factors belonging to group I have never been included experimentally in any single zygote, (though there is no evidence at present that all can not be so included). Not until it can be shown that more linked factors can be carried by a single gamete than half the number of chromosomes involved in the circle, can it be *certain* that two or more of the linked factors *must* lie in a single chromosome pair. Cleland suggests that a part of the linkage may be due to inclusion of genes in the same chromosome, and part to chromosomal cohesions. If this should prove true, an important question to be determined is what criteria can be used to distinguish between these two kinds of linkage.

Another difficulty for the chromosome-cohesion hypothesis is that the numerous 15-chromosome mutants from *Oenothera Lamarckiana* are characterized by marked differences in regard to nearly all features of the plant. De Vries and Boedijn (1923) have grouped these 15-chromosome forms into seven primary groups, corresponding with the seven haploid chromosomes, and have explained them on the basis of non-disjunction of a different chromosome for each group. If this plausible hypothesis is correct, each of the haploid chromosomes produces strikingly differential effects on many features of the plant. If the production of crossovers is due to an aberrant distribution of whole chromosomes, and each chromosome produces manifold effects, it seems reasonable to suppose that the crossover individuals would be differentiated by other characteristics besides the one which is being followed in any particular breeding problem. Except in the case of the lethal factors this has been, quite generally, not the case; as a rule the crossover individuals are quite indistinguishable from the non-crossovers, except in regard to the specific characteristic which differentiated the P_1 individuals.

The old-gold mutation appeared in a strain of *Oenothera Lamarckiana* which CLELAND (1925) has studied and has found to possess 12 chromosomes united into a circle, with one pair free. As *Lamarckiana* is demonstrably carrying, all the time, in heterozygous state, several factors of linkage-group I, it might be assumed that these linked factors are distributed among the several chromosomes of the circle, and that the *vetaurea* factor lies in the independent pair. But *Lamarckiana* has always shown independence of the factor for *brevistylis*, which is found to be likewise independent of old-gold. With only *one* independent pair of chromosomes there would be no provision for *two* independent factors. There remains the logical possibility, of course, that a *Lamarckiana* which simultaneously segregates out both old-gold and *brevistylis* might be found to possess only ten chromosomes in the circle, and two independent pairs. This is a point which needs to be investigated.

The demonstration that old-gold represents not alone an independent factor, but a member of a third linkage group (SHULL 1925), and the hint that *brevistylis* is likewise not merely an independent factor but a member of the second linkage group, suggest another line of work which may be expected to give a crucial test of the chromosome-cohesion hypothesis of linkage. Thus far, CLELAND has reported only one case (*Oe. biennis*) in which the chromosomes are grouped into more than one circle,—and in this case he assumes that, although they are grouped into two circles, they are distributed as if they were included in a single circle (CLELAND 1926). The finding of as many groups of cohering chromosomes as there are linkage groups present in any given case, would strongly support the cohesion hypothesis, if it be admitted that the circles are oriented on the spindle independently of each other. It is clear that a close coöperation between geneticist and cytologist may promptly result in a decision between the chromosome-cohesion hypothesis of linkage and the hypothesis that linkage results from the inclusion of factors in a single chromosome pair. If both of these cytological phenomena give rise to linkage, there may be as many linkage groups as the number of independent chromosomes plus the number of chromosome circles that are present, but no more. In other words there could be two linkage groups only in *Lamarckiana*, two in *biennis*, five in *rubrinervis*, seven in *blandina*, and so on. There is no evidence yet available that such differences exist in the number of linkage groups in the different taxonomic forms of Oenothera, but this may be due merely to the fact that we are still only at the beginning of an adequate study of linkage in this group.

SUMMARY

1. The new flower color, "old-gold" (mut. *vetaurea*) made its appearance in cultures of *Oenothera Lamarckiana* in 1921.

2. Appropriate crosses have demonstrated that it resulted from a gene mutation which probably occurred two years before the new characteristic became visible.

3. The old-gold factor, *v*, is recessive to yellow, and segregates from the yellow-flowered F_1 in a manner suggestive of a typical Mendelian recessive, but with considerable variation in the ratios.

4. The dominant yellow-flowered plants in the F_2 were found to consist of homozygotes and heterozygotes in about the typical Mendelian ratio, 1 : 2.

5. Old-gold is complementary to sulfur, the F_1 plants being always wild-type yellow.

6. In the F_2 from the cross between old-gold and sulfur, a fourth flower-color, gold-center (seg. *aurata*), appeared as the double recessive.

7. These results indicate that the four flower colors now known in Oenothera should be represented, when in the homozygous state, by the genotypic formulae: Yellow, $SSVV$; sulfur, $ssVV$; old-gold, $SSvv$; and gold-center, $ssvv$.

8. In some of the F_2 families the sulfur and gold-center classes were strikingly modified by the action of "gamete lethals," but in four families there was a close approach to the typical Mendelian dihybrid ratio, 9 yellow : 3 sulfur : 3 old-gold : 1 gold-center.

9. Tests of old-gold and sulfur segregates in F_2 families from the cross sulfur × old-gold showed that each of these types occurred in the heterozygous condition and in the homozygous condition in about the expected 2 : 1 ratio.

10. Analysis of the yellow-flowered group in an F_2 family, showed the presence of 1 *SSVV*, 3 *SSVv*, 4 *SsVV* and 8 *SsVv*, which is in essential agreement with the expected Mendelian ratio of 1 : 2 : 2 : 4.

11. The old-gold factor is independent in inheritance from the factor for sulfur and from the lethals and other factors associated with sulfur in linkage-group I.

12. It is also independent of *brevistylis* which has been used as the "marker" of linkage-group II.

13. It is, therefore, the first-discovered factor of linkage-group III, for which it may well serve as the "marker."

14. A brief critique is given of the hypothesis that linkage in the Oenotheras is based on the end-to-end fusion of non-homologous chromosomes to form circles (CLELAND).

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A METHOD FOR CALCULATING LINKAGE VALUES[1]

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INTRODUCTION

The calculation of linkage values in many plants is confined to F_2 distributions, due to difficulties in making backcrosses. EMERSON (1916), HALDANE (1919), and other authors have proposed methods by which the gametic ratio can be obtained from the observed phenotypic zygotic proportions and from this ratio the percentage of crossing over can be calculated. WOODWORTH (1916), COLLINS (1924), BRUNSON (1924) and others have modified or extended EMERSON's method for use in special cases, involving duplicate, complementary or supplementary factors.

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A study of the theoretical gametic ratios with their corresponding phenotypic zygotic ratios in a dihybrid F_2 distribution (table 1) has revealed some interesting relationships. Some of these relationships have already been worked out by the authors cited above, but most of those contained in this paper have not been published so far as I know. It seems desirable, therefore, to present these relationships together with additional formulae for measuring linkage intensities.

TABLE 1

Giving a number of different gametic series together with their corresponding theoretical F_2 *phenotypic series.*

GAMETIC SERIES				F_2 PHENOTYPIC SERIES				TOTAL (n)	CONSTANT (k)
r	s	s	r	AB	Ab	aB	ab		
10	1	1	10	342	21	21	100	484	14 611
9	1	1	9	281	19	19	81	400	14.48
8	1	1	8	226	17	17	64	324	14.320
7	1	1	7	177	15	15	49	256	14.125
6	1	1	6	134	13	13	36	196	13 877
5	1	1	5	97	11	11	25	144	13.555
4	1	1	4	66	9	9	16	100	13.12
3	1	1	3	41	7	7	9	64	12.5
2	1	1	2	22	5	5	4	36	11 555
1	1	1	1	9	3	3	1	16	10
1	2	2	1	19	8	8	1	36	8.888
1	3	3	1	33	15	15	1	64	8.5
1	4	4	1	51	24	24	1	100	8 32
1	5	5	1	73	35	35	1	144	8.222
1	6	6	1	99	48	48	1	196	8.163
1	7	7	1	129	63	63	1	256	8.125
1	8	8	1	163	80	80	1	324	8.098
1	9	9	1	201	99	99	1	400	8.08
1	10	10	1	243	120	120	1	484	8.066

SYMBOLS USED

The following symbols will be used:

r, the first and fourth member of the gametic series ($r=1$ in the repulsion phase).

s, the second and third member of the gametic series ($s=1$ in the coupling phase).

AB, Ab, aB and ab, theoretical values of the members of the F_2 phenotypic zygotic series.

AB_0, Ab_0, aB_0 and ab_0 observed values of the members of the F_2 phenotypic zygotic series.

n, the sum of AB, Ab, aB and ab.

n_0, the sum of AB_0, Ab_0, aB_0 and ab_0.

k, constant for a given series obtained by dividing the sum of AB and ab by n (or by dividing the sum of AB_0 and ab_0 by n_0) and multiplying the quotient by 16, (formulae 1 and 2).

E_0, the sum of AB_0 and ab_0.

M_0, the sum of Ab_0 and aB_0.

c, coefficient obtained by dividing n_0 by n.

THE CONSTANT (k)

As is shown in table 1, the value of the constant k is dependent on the relationship existing between the genetic factors concerned. If there is independent inheritance, k has a value of 10; if A and B are coupled, the value of k varies from 10 to 16, depending on the strength of the coupling; and if A and b are linked (repulsion phase), k varies from 10 to 8 depending on the strength of the linkage. If the linkage in the coupling phase is complete, k has a value of 16, but in case of complete linkage in the repulsion phase, k has a value of 8.

Figure 1 shows that as the value of r (coupling phase) increases, the value of the constant k also increases, approaching 16 as a limit. Figure 2 shows that as the value of s (repulsion phase) increases, the value of the constant k decreases, approaching 8 as a limit. Table 2 shows that when large values for r and s are assumed, the constant k approaches 16 in case of coupling and 8 in case of repulsion. With such high values for r and s the linkage is practically complete.

TABLE 2

Showing the value of k when the values of r (coupling) and s (repulsion) are extremely high.

GAMETIC SERIES				TOTAL (n)	CONSTANT (k)
r	s	s	r		
3199	1	1	3199	40,960,000	15.99500078125
1	3199	3199	1	40,960,000	8.00000078125

FORMULAE

The formulae given below are applicable when deviations from a dihybrid Mendelian F_2 distribution are caused by linkage. When such disturbances as differential viability of gametes or failure of many seeds of certain types to germinate are operating, the results obtained by the application of the formulae to observed values will be misleading. The following formula shows the method of obtaining the value of k from the theoretical F_2 phenotypic series:

$$k=\frac{16(AB+ab)}{n} \tag{1}$$

The general formula also holds for obtaining the value of k from observed F_2 phenotypic ratios:

$$k=\frac{16(AB_0+ab_0)}{n_0} \tag{2}$$

Most of the formulae that follow indicate the relationship of the members of *theoretical* series. Several formulae are given to show the relationship when *observed* values are used. All formulae which are applicable to observed values are applicable also to theoretical values, but formulae which are applicable to theoretical values are applicable to observed values only when the value of c is unity. The theoretical values can be calculated from the observed values by means of the constant k.

Formulae for finding the value of n when the value of r, s, AB, Ab, aB, ab, or k is known

Coupling phase

$$n=4(r+1)^2 \tag{3}$$

From formulae (15) and (17) the following value for AB is derived,

$$AB=\frac{n}{2}+\left(\frac{\sqrt{n}}{2}-1\right)^2 \tag{4}$$

Hence,

$$n=\left[\frac{2\sqrt{3AB-2}+2}{3}\right]^2 \tag{5}$$

$$n=(Ab+1)^2 \text{ or } (aB+1)^2 \tag{6}$$

$$n=\left[\,2(\sqrt{ab}+1)\right]^2 \tag{7}$$

$$n=\left[\frac{4(\sqrt{2k-16}+4)}{16-k}\right]^2 \tag{8}$$

Repulsion phase

$$n=4(s+1)^2 \tag{9}$$

$$n=2(AB-1) \tag{10}$$

$$n=4(Ab+1) \tag{11}$$

$$n=\frac{32}{k-8} \tag{12}$$

Formulae for finding the value of any member of either gametic or F_2 phenotypic series when the value of n is known

Coupling phase

$$r=\frac{\sqrt{n}}{2}-1 \tag{13}$$

$$AB=\frac{3n-4(\sqrt{n}-1)}{4} \quad \text{(derived from formula 5)} \tag{14}$$

$$AB=\frac{n}{2}+ab \tag{15}$$

$$Ab \text{ or } aB=\sqrt{n}-1 \tag{16}$$

$$ab=\left[\frac{\sqrt{n}}{2}-1\right]^2 \tag{17}$$

$$k=\frac{16\left[(\sqrt{n}-1)^2+1\right]}{n} \tag{18}$$

Repulsion phase

$$s=\frac{\sqrt{n}}{2}-1 \tag{19}$$

$$AB=\frac{n}{2}+1 \tag{20}$$

$$Ab \text{ or } aB=\frac{n}{4}-1 \tag{21}$$

$$k=\frac{8(n+4)}{n} \tag{22}$$

Formulae showing the relation of the different members of the phenotypic series to one another

Coupling phase

Substituting in formula 4, the value of n obtained in formula 6, we have

$$AB=\frac{(Ab+1)^2}{2}+\left[\frac{Ab+1}{2}-1\right]^2$$

which reduces to

$$AB=\frac{3\,(Ab)^2+2Ab+3}{4} \tag{23}$$

Substituting in formula 4, the value of *n* obtained in formula 7, we have

$$AB=\frac{[2(\sqrt{ab}+1)]^2}{2}+\left[\frac{2\sqrt{ab}+2}{2}-1\right]^2$$

which reduces to

$$AB=3ab+4\sqrt{ab}+2 \qquad (24)$$

$$Ab=\frac{2\sqrt{3AB-2}-1}{3} \text{ (derived from formula 23)} \qquad (25)$$

Substituting in formula 16, the value of *n* obtained in formula 7, we have

$$Ab=2\sqrt{ab}+1 \qquad (26)$$

Substituting in formula 17, the value of *n* obtained in formula 5, we have

$$ab=\left[\frac{\sqrt{3AB-2}+1}{3}-1\right]^2$$

which reduces to

$$ab=\frac{3AB-4\sqrt{3AB-2}+2}{9} \qquad (27)$$

Substituting in formula 17, the value of *n* obtained in formula 6, we have

$$ab=\left[\frac{Ab+1}{2}-1\right]^2$$

which reduces to

$$ab=\left[\frac{Ab-1}{2}\right]^2 \qquad (28)$$

Repulsion phase

$$AB=2Ab+3 \qquad (29)$$

Formulae for finding the percentage of crossing over when the value of r, s, AB, Ab, aB, ab, n or k is known

Coupling phase

$$\text{Percentage of crossing over}=100\left[\frac{1}{r+1}\right] \qquad (30)$$

From formulae 3 and 4 we derive

$$AB=\frac{4(r+1)^2}{2}+\left[\frac{2(r+1)}{2}-1\right]^2$$

From this equation we obtain

$$r=\frac{\sqrt{3AB-2}-2}{3} \qquad (31)$$

Substituting in formula 30, the value of r obtained in formula 31, we have

$$\text{Percentage of crossing over} = 100\left[\frac{3}{\sqrt{3AB-2}+1}\right] \tag{32}$$

$$r = \frac{Ab-1}{2} \quad \text{(derived from formulae 3 and 6)} \tag{33}$$

From formulae 30 and 33 we derive

$$\text{Percentage of crossing over} = 100\left[\frac{2}{Ab+1}\right] \tag{34}$$

$$r = \sqrt{ab} \quad \text{(derived from formulae 3 and 7)} \tag{35}$$

From formulae 30 and 35 we derive

$$\text{Percentage of crossing over} = 100\left[\frac{1}{\sqrt{ab}+1}\right] \tag{36}$$

$$\text{Percentage of crossing over} = 100\left[\frac{2}{\sqrt{n}}\right] \quad \text{(derived from formulae 3 and 30)} \tag{37}$$

From formula 8 we derive

$$r+1 = \frac{2(\sqrt{2(k-8)}+4)}{16-k} \tag{38}$$

Hence, $$\text{Percentage of crossing over} = 100\left[\frac{16-k}{2\sqrt{2(k-8)}+8}\right] \tag{39}$$

Repulsion phase

$$\text{Percentage crossing over} = 100\left[\frac{2}{\sqrt{2(AB-1)}}\right] \tag{40}$$

$$\text{Percentage crossing over} = 100\left[\frac{1}{\sqrt{Ab+1}}\right] \tag{41}$$

$$\text{Percentage crossing over} = 100\left[\frac{2}{\sqrt{n}}\right] \tag{42}$$

$$\text{Percentage crossing over} = 100\left[\frac{k-8}{2\sqrt{2(k-8)}}\right] \tag{43}$$

Formulae for calculating the percentage of crossing over from observed values

Coupling phase

Substituting in formula 39, the value of k obtained in formula 2, we have

$$\text{Percentage crossing over} = 100\left[\frac{\left(16-\dfrac{16(AB_0+ab_0)}{n_0}\right)}{2\sqrt{2\left(\dfrac{16(AB_0+ab_0)}{n_0}\right)-16}+8}\right]$$

which is equivalent to

$$\text{Percentage crossing over} = 100\left[1-\sqrt{\frac{AB_0-Ab_0-aB_0+ab_0}{AB_0+Ab_0+aB_0+ab_0}}\right]$$

Substituting E_0 for the sum of AB_0 and ab_0, M_0 for the sum of Ab_0 and aB_0, and n_0 for the sum of AB_0, Ab_0, aB_0 and ab_0, we have,

$$\text{Percentage of crossing over} = 100\left[1-\sqrt{\frac{E_0-M_0}{n_0}}\right] \tag{44}$$

Repulsion phase

Substituting in formula 43, the value of k obtained in formula 2, we have

$$\text{Percentage crossing over} = 100\left[\frac{\dfrac{16(AB_0+ab_0)}{n_0}-8}{2\sqrt{2\left(\dfrac{16(AB_0+ab_0)}{n_0}-8\right)}}\right]$$

which is equivalent to

$$\text{Percentage crossing over} = 100\sqrt{\frac{AB_0-Ab_0-aB_0+ab_0}{AB_0+Ab_0+aB_0+ab_0}}$$

Substituting E_0 for the sum of AB_0 and ab_0, M_0 for the sum of Ab_0 and aB_0, and n_0 for the sum of AB_0, Ab_0, aB_0 and ab_0, we have

$$\text{Percentage crossing over} = 100\sqrt{\frac{E_0-M_0}{n_0}} \tag{45}$$

The percentage of crossing over may also be obtained by dividing E_0 by M_0 and referring to table 3. Fractional percentages can be obtained by interpolation. As an example, the percentage of crossing over may be calculated as follows from the first distribution in table 4:

$$E_0=631; \qquad M_0=50$$

$$\frac{E_0}{M_0}=12.6$$

By referring to table 3 we find that when the value of $E_0 \div M_0 = 13.8038$ the percentage of crossing over is 7 and when the value of $E_0 \div M_0 = 12.0208$ the percentage of crossing over is 8. The difference $= 1.7830$

$$13.8038 - 12.6 = 1.2038$$

$$\frac{1.2038}{1.7830} \text{ of 1 per cent } = 0.67 \text{ per cent}$$

$$7.0 \text{ per cent } + 0.67 \text{ per cent} = 7.67 \text{ per cent}$$

TABLE 3

Crossing-over percentages from 1 to 50 and the corresponding ratios of E_0 *to* M_0.

COUPLING PHASE				REPULSION PHASE			
Percent crossing over	$\frac{E_0}{M_0}$	Percent crossing over	$\frac{E_0}{M_0}$	Percent crossing over	$\frac{E_0}{M_0}$	Percent crossing over	$\frac{E_0}{M_0}$
1	99.5025	26	3.4209	1	1.0002	26	1.1450
2	49.5051	27	3.2817	2	1.0008	27	1.1573
3	32.8409	28	3.1528	3	1.0018	28	1.1701
4	24.5102	29	3.0331	4	1.0032	29	1.1836
5	19.5128	30	2 9215	5	1.0050	30	1.1978
6	16.1822	31	2.8175	6	1.0072	31	1.2126
7	13.8038	32	2.7202	7	1.0098	32	1.2282
8	12.0208	33	2.6291	8	1.0129	33	1.2444
9	10.6346	34	2.5436	9	1.0163	34	1.2614
10	9.5263	35	2.4631	10	1.0202	35	1.2792
11	8.6200	36	2.3875	11	1.0245	36	1.2978
12	7.8652	37	2.3162	12	1.0292	37	1.3172
13	7.2271	38	2.2488	13	1.0344	38	1.3375
14	6.6805	39	2.1852	14	1.0400	39	1.3588
15	6.2072	40	2.1250	15	1.0460	40	1.3810
16	5.7935	41	2.0679	16	1.0525	41	1.4041
17	5.4288	42	2.0139	17	1.0595	42	1.4283
18	5.1050	43	1.9625	18	1.0670	43	1.4537
19	4.8157	44	1.9137	19	1.0749	44	1.4802
20	4.5556	45	1.8674	20	1.0833	45	1.5078
21	4.3206	46	1.8232	21	1.0923	46	1.5368
22	4.1073	47	1.7813	22	1.1017	47	1.5670
23	3.9128	48	1.7412	23	1.1117	48	1.5988
24	3.7349	49	1.7031	24	1.1222	49	1.6319
25	3.5714	50	1.6667	25	1.1333	50	1.6667

DISCUSSION

In order to illustrate the method of application of some of the formulae, table 4 was compiled. The data in this table are the same as those used by EMERSON (1916). Formula 2 was first used to obtain the value of k. From the value of k, the value of n was calculated by using formula 8 for the first two distributions and formula 12 for the third distribution. Finding the value of n in the coupling phase involves several computations because formula 8 is quite complex, but after n has once been obtained the calculation from n, of the other members of the zygotic series, is relatively simple. The value of k in the fourth distribution is 7.94. Since this value is less than 8, the repulsion formulae are not applicable. EMERSON (1916) found that the value of r for this distribution could not be determined by the formula which he proposed because $(1335+2)-(643+714)$ is a negative quantity (-20) and has no real root.

TABLE 4

Theoretical values of various terms obtained from observed values.

OBSERVED					THEORETICAL						
AB	Ab_0	aB_0	ab_0	n_0	k	r s	AB	Ab	aB	ab	n
493	25	25	138	681	14.825	12.1 : 1	488 8	25.2	25 2	146 1	685.3
165	58	58	78	359	10.830	1 46 . 1	14.30	3 93	3.93	2 13	24.34
336	150	143	11	640	8 675	1:2.44	24.7	10.85	10.85	1	47.4
1335	643	714	2	2694	7.94	..	.. ..	. .	. .	.	. .

The formulae reported in this paper are applicable only when the value of k is between 10 and 8 (repulsion phase) or 10 and 16 (coupling phase). With a reduction of the same number of AB_0 or ab_0 types for a given value of r or s the limits for the application of the formulae are reached sooner in the repulsion phase than in the coupling phase as is indicated by the nature of the curves in figures 1 and 2. In the coupling phase the value of the points on the curve approaches 16 *gradually*, while in the repulsion phase it approaches 8 *rapidly* at first, then more slowly.

The relationship of E_0 and M_0 to r and s is shown in the following formulae:

$$\sqrt{\frac{E_0-M_0}{E_0+M_0}}=\frac{r}{r+1} \qquad \text{(coupling phase)} \qquad (46)$$

and

$$\sqrt{\frac{E_0-M_0}{E_0+M_0}}=\frac{1}{s+1} \qquad \text{(repulsion phase)} \qquad (47)$$

The relationship between E_0 and M_0 may also be expressed as follows:

$$E_0=M_0+4cr^2 \qquad \text{(coupling phase)} \qquad (48)$$

and

$$E_0=M_0+4c \qquad \text{(repulsion phase)} \qquad (49)$$

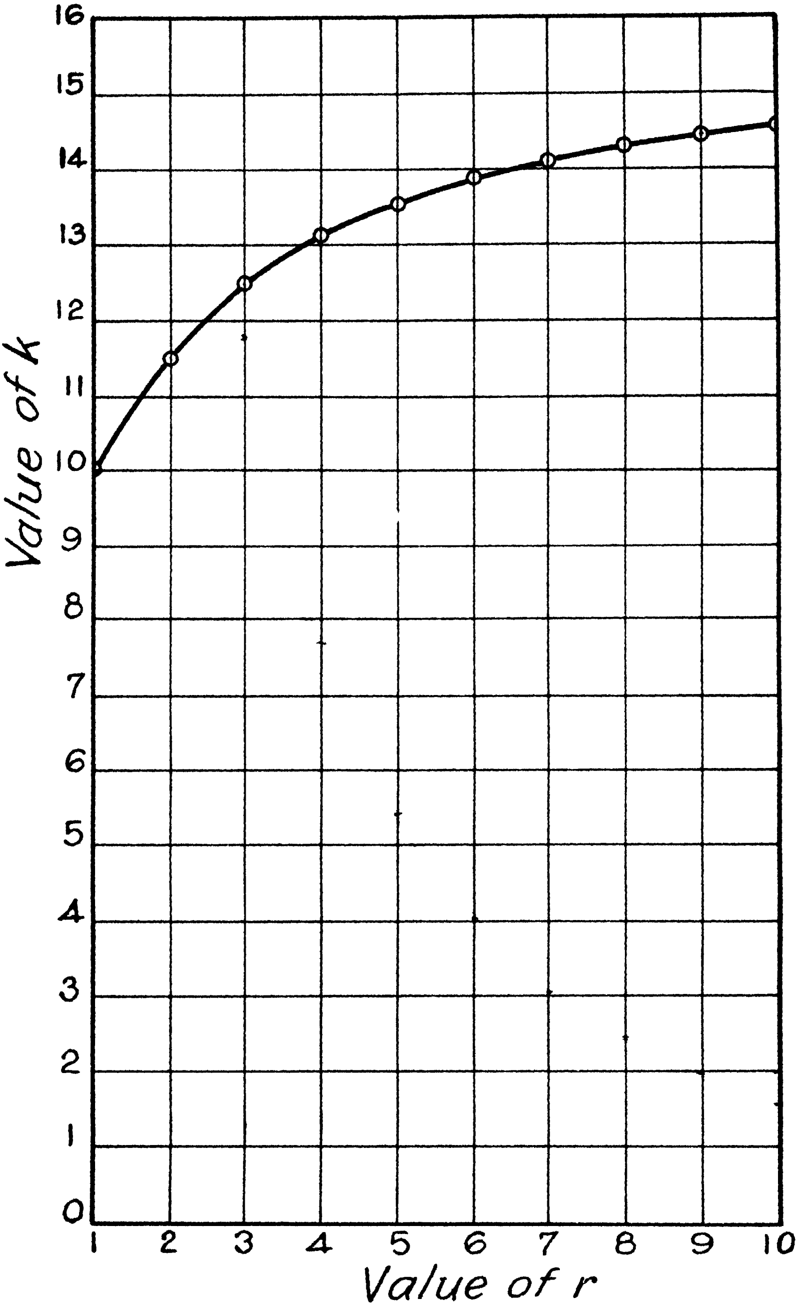

FIGURE 1.—The curve shows that when the value of r is increased, the value of k approaches 16 gradually.

A further relationship between E_0 and M_0 is indicated by formulae 50 and 51:

$$\frac{E_0}{M_0} = r + \frac{1}{2} + \frac{c}{M_0} \quad \text{(coupling phase)} \tag{50}$$

and

$$\frac{E_0}{M_0} = 1 + \frac{4c}{M_0} \quad \text{(repulsion phase)} \quad (51$$

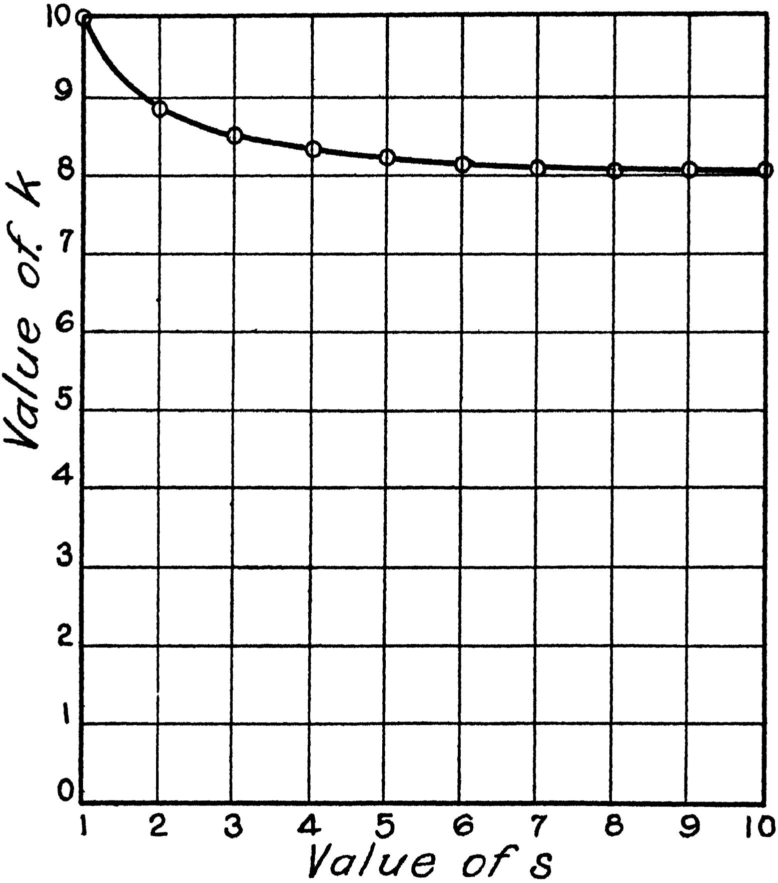

FIGURE 2.—The curve shows that when the value of *s* is increased, the value of *k* approaches 8, rapidly at first, then more slowly.

The value of E_0 divided by M_0 in the coupling phase is approximately equal to $r+\frac{1}{2}$ and the greater the magnitude of r the less is the magnitude of $\frac{c}{M_0}$ which approaches zero as the value of r approaches infinity. On the other hand, the value of E_0 divided by M_0 in the repulsion phase is approximately equal to 1, and the magnitude of $\frac{4c}{M_0}$ decreases as the value of s increases. The value of $\frac{4c}{M_0}$ approaches zero as the value of s approaches infinity. In other words, the ratio of E_0 to M_0 is approximately equal to the magnitude of $r+\frac{1}{2}$ in the coupling phase while in the repulsion phase E_0 is approximately equal to M_0. With this relationship in mind it is evident that a considerable reduction of AB_0 and ab_0 types, caused perhaps by differential viability or selective fertilization, is permissible in the coupling phase to meet the conditions necessary for the application of the formulae. The effect of such reductions, however, is not corrected by the formulae and thus an erroneous value for r is obtained. On the other hand, owing to the approximate equality of E_0 and M_0 in the repulsion phase, a slight reduction of the AB_0 and ab_0 types may cause the value of E_0 to be less than M_0. If the reduction of these types is greater than $4c$, then E_0 minus M_0 will have a negative value.

In order that the formulae may be usable, the observed values should deviate as little as possible from the result obtained by multiplying the theoretical distribution by c. If the observed distribution corresponds to this distribution, the quotient obtained by dividing the sum of the types AB_0 and Ab_0 by the types aB_0 and ab_0 is a constant, being equal to 3 in both coupling and repulsion phases.

$$\frac{AB_0+Ab_0}{aB_0+ab_0}=3 \tag{52}$$

SUMMARY

1. A constant k which shows the relation of observed values in linkage studies to independent inheritance is obtained.

2. The value of k indicates the following:
    (a) When $k=10$, independent inheritance.
    (b) When $k=$ more than 10 but less than 16, coupling.
    (c) When $k=$ less than 10 but more than 8, repulsion.
    (d) When $k=$ less than 8, some factor other than chance resulting in marked deviations from theoretical ratios.

3. Formulae are given showing the relation of the members of the theoretical gametic and zygotic series to one another.

4. The relationship between the extreme terms and the middle terms of a distribution is shown.

5. Formulae and tables for determining the percentage of crossing over are presented.

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THE LINKAGE RELATIONS OF A BENIGN TUMOR IN DROSOPHILA

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INTRODUCTION

It has already been reported (STARK 1919) that the "benign" tumor which appeared as a mutation in the lethal-tumor strain of flies (lethal 7) is due primarily to a recessive gene located in the third chromosome close to dichaete. This conclusion was based on the results of a backcross between the tumor and dichaete and hairless (P_1, $D\ H$ ♀ × t_u ♂; F_1, $D\ H$ ♀ × t_u ♂) which gave: $D\ H=348$, wild-type $=276$, $D=108$, $H=78$, $t_u=30$, $t_u\ H=10$. Instead of 50 percent, only 5 percent of the flies in the backcross showed the tumor character. This low value indicated that more than one recessive gene was concerned in the production of the tumor. In order to locate more exactly the principal gene in the third chromosome and also to locate the other genes that appeared to be involved, the same methods were employed that had been used in analyzing the complex inheritance of vortex (BRIDGES and MOHR 1919) and of ski (CLAUSEN and COLLINS 1922).

STAR DICHAETE × TUMOR, MALE BACKCROSS

From the pure-breeding stock of tumor flies two males were mated, each to a star dichaete female. Star is a second-chromosome dominant located at the extreme left end of the second-chromosome map (0.0). Dichaete is a third-chromosome dominant located somewhat to the left of the middle of the third chromosome (40.4). The F_1 star dichaete males were then mated separately, each to a virgin female from the tumor stock. (In table 1, Nos. 11 to 18 are from one P_1 mating, and Nos. 31 to 36 are from the other.) The flies of the "backcross" generation were classified with respect to star, dichaete and tumor.

When the results for the different backcross cultures were examined, it was seen that there was a very striking diversity, not only in the percentages of tumor flies (in the last column), but also in the frequency with which star and dichaete flies bore tumors. This signified that the tumor stock or the star dichaete stock or both had not been homozygous with respect to the genes affecting tumor development. If the stocks had been uniform, all of the backcross cultures should have been of a single type, though differences due to errors of random sampling might be present. A serious source of difference in the use of certain characters arises from their extreme sensitivity to slight and uncontrolled environmental differences, such as quality and condition of the food materials. Thus, a high proportion of the Drosophila mutant characters show a progressive "changing over" toward the wild type as the flies emerge later and later in the course of the culture. This can be largely corrected by the occasional addition of fresh food early in the course of the culture, but an extended examination of the cultures producing tumors suggests that the differences in the degree of development of the character (the percentage of individuals that show the character) are in the main dependent on genetic causes rather than environmental. In the experiment whose results are given in table 1 the cultures fell into four types, each sharply different from the others, and the constituent members of a given group resembled each other closely.

The most frequent type of culture that occurred in the star dichaete ×tumor male backcross was characterized by complete or nearly complete absence of tumors in flies that were dichaete or dichaete with star (table 1, upper division). This showed that the genes (or gene) having the greatest effectiveness in the production of the tumors were recessives in the third chromosome. That is, a fly in those cultures could not develop tumors unless both third chromosomes were derived from the tumor stock, one directly from the tumor-bearing mother and the other through the F_1 star dichaete male. The third chromosome from the tumor stock that was present in the F_1 *S D* ♂ was kept intact because there is no crossing over in a male, and was transmitted to all of the offspring that did not show the dominant character dichaete.

But the fact that a fly was homozygous for the entire third chromosome from the tumor stock did not insure that tumors develop in it. Thus, of the 631 flies, (of the upper division of table 1) that were not-dichaete, and that therefore had both their third chromosomes from the tumor stock, 343 or 54 percent had tumors, but the remainder, or 46 percent, did not have tumors. These 46 percent of flies were non-tumor-bearing because some other gene or genes besides the third-chromosome recessives were necessary to insure the development of the tumor character. That these other genes were predominantly in the second chromosome was shown by the fact that among the not-dichaete flies that were star, some (91) were tumored but many more (230) were non-tumored.

TABLE 1

P_1, star dichaete ♀ × tumor ♂, backcross tu ♀ × F_1SD ♂

GROUP	CULTURE NUMBER	+	*tu*	*S*	*S tu*	*D*	*D tu*	*S D*	*S D tu*	PERCENT *tu*
I	11	3	37	32	13	42		50		29
	15	3	38	21	7	34		34		33
	31		9	10	4	11		20		24
	32	25	87	78	39	84		103	1	30
	35	15	54	47	14	71		70	2	25
	36	12	27	42	14	42		65		30
Totals		58	252	230	91	284		342	3	27
II	13	4	22	2	12	18	2	24		43
	14	1	12	3	9	13		13		41
	17	6	31	15	21	37		31		37
	33	6	17	9	12	19		17		36
Totals		17	82	29	54	87	2	85		40
III	12	5	13	23	2	21	6	19	1	25
	16	5	20	29	5	12	12	30		33
Totals		10	33	52	7	33	18	49	1	29
IV	18	2	19	6	11	5	12	10	5	66

A closer analysis of the effectiveness of the mutant genes involved could be made by studying the percentage of occurrence of tumors in the different classes with respect to star and dichaete. Thus, the class *S D* differed from wild flies by being heterozygous for any tumor genes carried in chromosomes II and III of the tumor stock. Of the *S D* flies in group I only 3 bore tumors to 342 that did not. That is, the dominant effect of the combined second and third chromosome genes upon production of tumors was negligible. The flies that were dichaete but not star differed from the *S D* flies in that they were homozygous for the second-chromosome genes of the tumor stock. Of the *D* flies none bore tumors, while 284 were without tumors. As pointed out before, this meant that homozygosis for second-chromosome genes was ineffective if the fly were only heterozygous for the main tumor genes, which were recessives in the third chromosome. The *S* not-*D* flies differed from the *S D* flies in that they were homozygous for third-chromosome genes. Of the *S* flies 91 were tumor-bearing to 230 that were free from tumors. That is, homozygosis for the third-chromosome genes produced tumors in about a quarter of the flies that were only heterozygous for the second-chromosome genes. Conversely, the second-chromosome genes were not essential in a homozygous form, for 91 flies merely heterozygous for the second-chromosome genes were made to produce tumors by the action of the third-chromosome gene. This agreed with the previous comparison (of frequency of tumors in all *D* as compared with all not-*D* flies) in showing that the third-chromosome genes were responsible for a greater share of tumor development than were the second-chromosome genes. A comparison of the not-*S* not-*D* class with the *S* not-*D* class showed that in the presence of homozygous III tumor genes, homozygous II tumor genes raised the percentage of tumors from 28 to 81. This showed a considerable effectiveness of recessive second-chromosome genes, accounting for nearly all of the effectiveness not already accounted for by third-chromosome recessives. Finally, in flies that were homozygous for chromosome II and at the same time for chromosome III (that is, that were neither star nor dichaete) 352 were tumored and 58, or 19 percent, were non-tumored. Since all of the flies of the parental tumor stock were tumor-bearing, it follows that flies that were homozygous for both the second and third chromosomes and yet failed to develop tumors (19 percent) lacked certain tumor genes that were present in the parental stock. The locus of these other genes, of relatively slight aggregate effect, must be sought in the other chromosomes. —the X and chromosome IV.

The second group of backcross cultures (table 1, second division) agreed with the first in that practically none of the flies that were dichaete was at the same time tumor-bearing, but differed in that the star (not-dichaete) flies with tumors (54) outnumbered the star (not-dichaete) flies without tumors (29). In the first group the incidence of tumors in the star not-dichaete flies was 28 percent, while it was 65 percent in the second group. Moreover, the percentage frequency for each culture was higher than in the first group, giving an average of 40 percent instead of 27 percent. The increase from 28 to 65 percent of tumors in the star class, and the increased frequency of tumors from 27 to 40 percent per culture, could be accounted for either by assuming that additional third-chromosome recessive genes were present in group II cultures, or that there were additional second-chromosome genes that were dominant or semidominant.

The third group of cultures comprised two only, which agreed with the first group with respect to the percentage frequency of tumors in the star (not-dichaete) class, but differed in that a considerable percentage of the (not-star) dichaete flies were tumor-bearing (18÷51 or 35 percent, as compared with 0÷284 in group I). This difference could be attributed either to additional second-chromosome recessives, or, more probably, to additional third-chromosome dominants.

One culture was present (group IV) that apparently combined the additional modifiers present in group II with the additional modifiers present in group III. Thus, in the star class 65 percent of the star flies were tumor-bearing, which agreed with the 65 percent of group II. In the *S D* class 35 percent of the flies were tumor-bearing, showing conclusively the presence of dominant modifiers, and agreeing with the hypothesis advanced for group III. Two additional sets of modifiers also resulted in an increase in the total tumor frequency from the 27 percent of group I to 66 in group IV.

In all of the four groups the percentage of non-tumored flies in the not-star not-dichaete class (columns 1 and 2) was approximately constant, with an average of 18 percent (I = 19, II = 17, III = 23, IV = 10). That is, the tumor stock used, that gave 100 percent of tumor-bearing flies, differed from the non-tumored star dichaete parent stock in genes outside of the controlled chromosomes II and III to the extent of 18 percent of the total tumor production. Apparently the stocks were uniform with respect to these other genes, located in the X and IV chromosomes. The stocks were also uniform with respect to the second- and third-chromosome genes responsible for the results of group I, and present in the other groups. The stocks contained, but were not uniform for, additional second- and third-chromosome modifiers responsible for group II, and for group III, and, acting together, for group IV.

STAR DICHAETE × TUMOR, FEMALE BACKCROSS

From the crosses of star dichaete × tumor that gave the F_1 males tested in table 1, star dichaete F_1 females were mated each to a male from the tumor stock. The cultures (table 2) were not markedly different from one another, ranging in percentage of tumors from 10 to 21. Three subtotals have been calculated: for the lowest three cultures; for the group having 16 to 17 percent of tumors; and for the group having 20 or 21 percent of tumors. The grand total gives 16.3 percent of tumors, which was lower than the percentage (27) given in group I of table 1. Most of the decrease occurred in the not-star not-dichaete class, in which there were 588 tumor-bearing to 1479 normal flies. The percentage of tumored flies in this class was 35 as compared with 81 percent in the same class in the reciprocal backcross.

TABLE 2.

P_1, star dichaete ♀ × tumor ♂; backcross, F_1 S D ♀ × t_u ♂.

CULTURE NUMBER	+	*tu*	*S*	*S tu*	*D*	*D tu*	*S D*	*S D tu*	PERCENT *tu*
4	70	28	81	12	127	5	117	1	10
3	137	58	159	21	151	5	174	3	12
1	101	40	108	28	138	7	130	1	14
Totals	308	126	348	61	416	17	421	5	12
2	123	52	121	48	195	16	146	3	16
5	85	51	123	28	148	5	138	8	16
6	104	38	118	36	128	7	100	4	16
7	56	30	60	17	75	6	86		16
9	68	45	68	21	123	3	87		16
30	46	26	55	14	76	5	66	1	16
29	43	27	52	13	44		61	1	17
Totals	525	269	597	177	789	42	684	17	16
8	33	32	47	13	53	5	65		20
26	77	52	93	26	104	11	94	4	20
27	56	37	81	30	86	4	78		20
28	41	32	39	13	55	2	54	4	21
25	42	40	51	22	64	1	83	2	21
Totals	249	193	311	104	362	23	374	10	20
Grand Totals	1082	588	1256	342	1567	82	1479	32	16

Such a difference was to be expected if there were in the second chromosome more than one gene favoring tumor development, and likewise in the third. In the crosses of table 1 the several tumor genes in a given chromosome were held together by the lack of crossing over in the male, and consequently, could give a maximum number of favorable combinations. In the female, crossing over occurs and allows the tumor genes in a given chromosome to be separated, so that the number of favorable combinations is decreased, and is the smaller the higher the crossing over, that is, the farther apart within the chromosome the various genes lie. The observed decrease was taken to demonstrate that the effect due to the second or the third chromosome or to both was an aggregate one and not due to a single gene in each.

While crossing over tended to decrease the total production of tumors, it tended to increase the proportion of tumors that occurred with star and with dichaete. Thus, of the 1044 flies with tumors, 374 or 36 percent were with star, as compared with 27 percent with star in group I of the male backcross. Likewise 11 percent tumors were with dichaete as contrasted with 1 percent in the reciprocal backcross. The results with dichaete suggest that for the third chromosome most of the effect is due to a main gene located roughly 10 units from dichaete. For the second chromosome the effect is more widely distributed, but little can be said as to the location of the particular genes.

From table 2, a few females of the star dichaete class were mated singly to males of the tumor class (table 3). Three of the cultures gave percentages of tumors like those of table 2, but the other three gave markedly higher tumor percentages. In these last-mentioned cultures the mothers had become homozygous for certain tumor genes, either those outside chromosomes II and III, which involved simply assortment, or

TABLE 3

S D ♀ (*from table 2*)$\times t_u$ ♂ (*from table 2*)

CULTURE NUMBER	+	*tu*	*S*	*S tu*	*D*	*D tu*	*S D*	*S D tu*	PERCENT *tu*
20	61	37	80	11	86	8	99	3	15
22	73	31	65	12	53	7	58	2	17
21	34	19	33	12	34	3	35	5	22
19	79	31	85	55	103	8	79	37	27
23	29	36	36	38	54	17	64	10	36
24	22	37	26	24	32	10	42	9	40
Totals	298	191	325	152	362	53	377	66	25

TABLE 4

P_1, tumor ♂×dichaete hairless ♂; backcross, F_1 D H ♀×t_u♂.

GENERATION	CULTURE NUMBER	*D H*	+	*D*	*H*	*tu*	*tu D H*	*tu H*	*tu D*	PERCENT *tu*
BC	37	181	229	59	48	39	12	3	..	9
	38	155	235	68	52	27	2	4	.	6
	39	69	53	28	18	16	3	5	1	12
	40	81	109	31	25	13	1	5		7
	41	77	86	17	31	7	5	..	..	5
	Totals	563	712	203	174	102	23	17	1	8
BC$_2$	42	27	43	13	12	10	3	3	1	15
	43	102	142	50	38	4	..	3	2	2
	44	67	62	16	9	16	2	2	5	14
	45	54	93	26	5	25	6	2	..	15
	46	43	53	21	8	29	28	8	11	37
	47	76	63	29	12	54	17	7	10	33
	48	119	126	57	37	33	24	9	6	17
	Totals	488	582	212	121	171	80	34	35	19
BC$_3$	49	44	59	26	13	30	8	6	2	20
	50	30	14	15	4	21	16	10	6	46
	51	52	37	29	22	30	7	13	1	26
	52	34	51	29	13	56	33	25	10	50
	53	48	65	23	18	15	6	5	..	15
	Totals	208	226	122	70	152	70	59	19	32
BC$_4$	54	34	70	20	21	12	6	9	4	17
	55	33	33	30	11	17	14	9	3	28
	56	31	27	13	7	44	27	12	10	50
	Totals	98	130	63	39	73	47	30	17	35
BC$_5$	57	36	31	10	6	22	20	3	7	43
	58	52	52	19	10	33	2	5	1	18
	59	36	35	19	7	20	8	16	2	32
	60	27	29	6	5	33	35	4	9	55
	Totals	151	147	54	28	108	65	28	19	36
BC$_6$	61	5	15	2	4	10	9	6	5	55
	62	23	31	10	16	23	15	18	7	44
	63	28	22	4	3	37	18	11	4	55
	64	10	15	2	3	36	18	6	7	70
	65	69	56	25	13	17	11	5	7	19
	66	16	10	4	2	27	4	1	1	56
	67	27	30	8	7	70	35	16	16	63
	Totals	178	179	55	48	220	110	63	47	48
BC$_7$	68	10	32	10	4	26	45	10	7	61
	69	16	21	7	4	24	34	8	8	60
	70	26	39	12	7	42	36	21	19	58
	Totals	52	92	29	15	92	115	39	34	60
Grand totals		1738	2068	738	495	918	510	270	172	27

for some of those in chromosomes II and III, which involved crossing over. In culture 19, 70 percent of the tumors were with star, showing that the star chromosome had received by crossing over a very effective tumor gene.

DICHAETE HAIRLESS×TUMOR, FEMALE BACKCROSS

The backcross of tumor to star and dichaete had showed that there was a main gene for tumor located in the third chromosome roughly ten units distant from dichaete. To determine this distance more accurately and also to find whether the locus of the tumor gene was to the left of dichaete at roughly 30 or to the right at roughly 50, a backcross was made involving tumor and the dominant hairless in addition to dichaete. The locus of hairless is about 30 units to the right of dichaete (that is, at 69.5).

In the backcross results (cultures 37 to 41, table 4) only one tumor dichaete fly occurred, while 23 tumor dichaete hairless flies occurred. The relative difficulty in the formation of the tumor dichaete class indicated that it was the class due to double crossing over, and hence, the locus of tumor was to the left of dichaete and still farther to the left of hairless. The percentage of recombination for tumor and dichaete, figured on the basis of the tumor-bearing flies, was 17, a value somewhat higher than the 10 found in the star dichaete×tumor backcross. In the $D\ H \times t_u$ results only 8 percent of the flies bore tumors, and that again showed that tumor was a polygenic character.

Backcrosses were continued for several generations by crossing *D H* females from one of the cultures giving the highest percentages of tumors, to tumor brothers (table 4). In each successive generation the average percentage of tumors rose, reaching 60 in the seventh backcross generation (BC_7). The various frequencies of tumors represent the various combinations of tumor genes, with increasing homozygosis.

In table 5 the cultures have been collected according to the percentage of tumors shown. A comparison of the groups shows an increasing percentage frequency of tumors with dichaete, showing that certain of the tumor genes that have become homozygous were in the third chromosome. But the increase in tumors with dichaete was not as rapid as the general increase in tumor frequency, which indicated that genes outside chromosome III were likewise becoming homozygous. The recombination for dichaete and hairless did not vary greatly, and the average percentage for the grand total was 24.3, which was close to the standard value of 25.7.

TABLE 5
Cultures of table 4 grouped according to percentage of tumors.

GROUP	*D H*	+	*D*	*H*	*tu*	*tu D H*	*tu H*	*tu D*	MEAN PERCENT	*tu-D* RECOMBINATION	*D-H* RECOMBINATION
0–10	596	801	225	194	90	20	15	2	7	17	22
10 20	539	620	227	143	177	63	45	25	17	28	24
20–35	241	227	133	65	151	54	51	18	29	25	28
35–55	197	207	98	54	195	139	76	51	45	41	27
55–70	165	213	55	39	305	234	83	76	60	44	22
Total	1738	2068	738	495	918	510	270	172	27	36	24.3

ROUGHOID TUMOR×DICHAETE, FEMALE BACKCROSS

The dichaete hairless×tumor backcross had given the locus of the main third-chromosome tumor gene at about 17 units to the left of dichaete. The value 17 was based on rather small numbers, and was in disagreement with the value 10 found in the star dichaete×tumor backcross. The locus of tumor should, in any case, be rather close to that of hairy, which is about 15 units to the left of dichaete. But the uncertainty in the order of loci, and the closeness of the loci together would make it very difficult to obtain a double recessive of hairy with tumor. Instead, the locus of tumor was checked by the use of roughoid, which lies at the zero of the third chromosome. A tumor-bearing female was mated to a male that was roughoid hairy scarlet peach spineless sooty, and the F_1 wild-type flies were inbred. From the F_2 flies those were selected that showed roughoid but none of the other third-chromosome characters. Such flies resulted from crossing over between roughoid and hairy, and should carry with roughoid all of the tumor chromosome that lay to the right of the point of crossing over. When the F_2 roughoid flies were inbred, a few flies were found in F_3 that showed both roughoid and tumor. Some of these were bred together, giving a roughoid tumor stock, which, however, threw only about 15 percent of tumors. Dichaete females were crossed to roughoid tumor males, and the F_1 dichaete females were crossed to roughoid tumor males from the stock (upper division of table 6). In the backcross only a very few tumor flies appeared, about 2 percent of the total (totals by generations given in table 7). Such a low value was in conformity with the known polygenic nature of the tumor character, and this value was lower than that in the dichaete hairless×tumor backcross, because the roughoid tumor stock used was one that did not contain the full complement of tumor genes. The few tumor flies that did appear in the backcross were distributed nearly at random with respect to roughoid and dichaete, and no conclusions as to order and distance could be safely drawn.

TABLE 6

P_1, *dichaete* ♀ × *roughoid tumor* ♂; *backcross* F_1D ♀ × $ru\ t_u$ ♂.

GENERATION	CULTURE NUMBER		*D*	*D ru*	+	*ru*	*ru tu*	*tu*	*ru tu D*	*tu D*	PERCENT *tu*
BC		71	50	19	36	18		1	1	.	2
		72	84	20	30	24		1	.	2	2
		73	56	17	51	38	. .	1	.	.	1
		74	44	21	35	40	2			1	2
		75	88	18	98	7	3	1		1	2
		76	46	41	47	57	1	.	1	1	2
BC_2	71	77	146	80	90	133	12	2			3
		78	102	46	80	113	1	2		1	1
	72	79	98	60	78	90	1			.	0
		80	48	33	32	34				2	1
		81	122	64	81	95	1	2		1	1
		82	82	45	32	43	17	1	2		9
		83	72	18	74	28		.		1	1
	74	84	66	30	26	36	14	7	5		14
		85	86	64	44	65	37	13	4	2	18
		86	94	46	51	74	12	10	3	1	9
		87	116	37	28	87	36	12	2	2	16
		88	85	49	65	73	25	5	4	1	11
		89	44	22	35	34	10	7	4	1	14
	76	90	75	66	72	70	7	6			4
BC^3	77	91	192	98	96	128	56	16	9		14
	82	92	94	56	45	27	52	27	12	2	30
		93	130	93	76	64	80	21	18	2	25
		94	145	76	79	83	42	22	7	3	16
		95	133	92	97	88	53	20	7	.	16
	84	96	110	67	71	84	38	15	9	3	16
	85	97	234	113	128	120	61	33	10	5	15
		98	31	12	21	14	35	6	6	2	39
	86	99	239	103	141	161	73	33	22	4	17
BC_4	93	100	35	15	25	14	22	12	4	.	30
	96	101	15	17	15	8	28	10	1	1	42
		102	122	52	45	26	108	43	17	4	41
		103	75	38	27	24	42	27	13	2	34
		104	70	29	28	12	63	25	16	5	44
		105	89	44	48	16	86	33	40	2	45
	97	106	129	80	72	81	32	21	10	1	15
	99	107	90	56	57	24	70	27	17	3	34
		108	19	15	7	10	23	9	1	1	40

TABLE 6 (continued)

GENERATION	CULTURE NUMBER		*D*	*D ryu*	+	*ru*	*ru tu*	*tu*	*ru tu D*	*ru D*	PERCENT *tu*
BC$_5$	102	109	163	66	50	33	148	59	29	9	44
		110	95	30	43	36	94	21	12	4	38
		111	72	29	18	17	50	16	16	4	39
	107	112	151	79	68	52	100	43	23	7	33
		113	156	88	76	68	100	42	28	3	31
BC$_6$	109	114	201	93	60	37	182	83	38	7	44
		115	64	28	34	10	62	38	16	6	47
		116	124	58	54	10	123	50	33	5	46
		117	149	83	50	43	84	34	19	4	30
	112	118	137	87	68	53	111	54	31	16	38
	113	119	39	15	28	4	53	31	9	2	52
		120	44	30	28	6	53	15	9	.	42
		121	28	14	9	6	22	8	8	..	40

TABLE 7

Cultures of table 6 totaled according to generations.

GENERATION	*D*	*D ru*	+	*ru*	*ru tu*	*tu*	*ru tu D*	*tu D*	PERCENT *tu*	*ru-tu*	*tu-D*	*ru-D*
BC	368	136	297	184	6	4	2	5	2	..	..	33
BC$_2$	1246	660	788	975	173	67	24	12	7	29	13	39
BC$_3$	1308	710	754	769	490	193	100	21	18	27	15	40
BC$_4$	644	346	324	215	474	207	119	19	35	28	17	42
BC$_5$	637	292	255	206	492	181	108	27	37	26	17	38
BC$_6$	786	408	331	169	690	313	163	40	42	28	17	42
Totals	4979	2552	2749	2518	2325	965	516	124	24	27.7	16.3	40.5

From some of the backcross cultures dichaete females and roughoid tumor males were mated for another generation (BC$_2$). In this generation about half of the cultures gave the same low percentages of tumors as occurred in the first backcross, but the others gave from 9 to 18 percent of tumors. These cultures evidently represent a new level of tumor production due to the combination in the parents of more tumor genes than were together in the original backcross. The distribution of tumors with respect to roughoid and dichaete became significant with the lowest class, tumor dichaete—that due to double crossing over. The order of loci was thus roughoid, tumor, dichaete, as expected from the results with dichaete hairless.

Four more generations were raised, using parents from the cultures in which the best combinations of tumor genes were present. The percentage of tumors rose by steps, but the r_u-t_u and the t_u-D recombination percentages remained nearly constant, with averages for the whole data of 27.7 and 16.3. The sum of these two values is 44, which is 3.6 points higher than the standard r_u-D crossing over total of 40.4. Evidently, crossing over was a little higher in these stocks than in standard stocks. If the locus of tumor is calculated as to its proportionate location on a standard map, it is found to be 15 units to the left of dichaete, or at 25.4.

The cultures of table 6 were plotted according to the percentage of tumors, and they were found to fall into four groups. Thirteen cultures showed percentages below 5, mostly with 2 percent of tumors. Then, between 5 and 9 there were no cultures. A second group had a mean at 15, a mode at 16, and a range from 9 to 19. An isolated culture at 25 has been included with the third group, whose mean was at 31, and range, outside the one at 25, from 30 to 35. The fourth group had a mean at 43 and a range from 38 to 47 with an extreme culture at 52. The cultures in each of the four groups were totaled (table 8) and the percentages of recombination calculated. In the group whose range was from 0 to 5, the incidence of tumor was very irregular, suggesting that with so low a genetic basis for tumor production, environmental factors were relatively more effective in influencing the result. In the remaining three groups the recombination percentages were constant, showing that the rise in percentage of tumors was mainly due to the accumulation of tumor genes in other chromosomes than the third, and possibly in the third but in the region to the right of dichaete.

TABLE 8

Cultures of table 6 grouped according to percentage of tumors.

GROUP	*D*	*D ru*	+	*ru*	*ru tu*	*tu*	r^u *tu D*	*tu D*	PERCENT *tu*	*ru-tu*	*tu-D*	*ru-D*
0-5	1031	503	804	747	28	16	2	10	2	46	21	42
9-19	1765	922	965	1154	506	215	98	23	15	28	14	41
25-35	880	508	424	316	550	233	134	23	31	27	17	42
38-53	1313	619	556	298	1241	501	282	68	43	27	17	40

TUMOR × BLACK PURPLE

The star dichaete × tumor backcross had shown that there was free recombination of star and tumor, and therefore, that the locus of the principal second-chromosome tumor gene was probably in the region near, or to the right of, black. Since there were at that time no usable dominant characters in the region to the right of black the location must be determined by using two recessives. According to the star tumor recombination percent the tumor gene might lie rather near black, and so black and purple seemed as far to the right as would be safe to use. To secure a stock of black purple with tumor, tumor females were mated to black purple vestigial males. F_2 flies that were black and purple but were not-vestigial were mated together. Such flies had the right end of the chromosome replaced, through crossing over, by the corresponding right end of the second chromosome of the tumor stock. In F_3, a few black purple tumor flies appeared, which were inbred to give a stock.

In the roughoid tumor×dichaete backcross the percentage of tumors in the first backcross generation had been very low and the distribution of tumors not significant. This meant that the data were nearly useless in the problem of finding the locus of the gene. In subsequent generations more tumor genes outside the region under test were brought together and the data of these later generations were satisfactory. In order to secure, in the first place, a favorable combination of tumor genes outside the region under test, tumor flies were mated to vestigial females. The vestigial flies that appeared in F_2 might have first-, third- and fourth-chromosome tumor genes, but were prevented from having second-chromosome genes by the selection of vestigial, which marks the non-tumor second chromosome. Crossing over might introduce into the vestigial chromosome tumor genes to the right or to the left of vestigial, but the star×tumor backcross had shown that there were probably no very important genes to the left of black, and not a great proportion of the vestigials would be crossovers to the right of vestigial. By starting from several F_2 vestigials, the type of result given by the majority of lines would be that in which crossing over had not occurred. The "B C" cultures of table 9 were from four such lines; and since they were roughly similar, it is probable that they were all of the non-crossover type. The percentage of tumors was high even in the first backcross generation. The recombination of tumor with black and purple was very free, as shown by the large number of not-black not-purple tumor flies and also by the numerous black purple not-tumor flies. Hence, the locus of the tumor gene must have been very far to the right of black and purple, probably close to speck in the extreme right end. No close calculation of the position could be made from the data, since it became apparent that the difficulty of detecting tumors in black flies was much greater than in not-black flies. This fact explained the $b\ p_r$ class being seemingly greater than the $b\ p_r\ t_u$ class, a high proportion of the flies that were actually tumor-bearing were classified as non-tumored. Two subsequent back-

TABLE 9

P_1, *wild* ♀×*black purple tumor* ♂; *backcross*, F_1+ ♀×*b* p_r t_u ♂.

GENERATION	CULTURE NUMBER		*b pr*	*b pr tu*	+	*tu*	*b*	*b tu*	*pr*	*pr tu*	PERCENT *tu*
BC$_1$		200	77	73	57	77	1	.	1	1	53
		201	62	20	21	31	2	.	5	2	37
		202	31	35	31	29	2		3	1	49
		203	97	13	90	18			2	.	14
		204	28	52	29	41	.		1	1	62
		205	51	32	58	48	1	.	1	1	42
		206	34	22	33	32	3		12	3	41
		207	33	15	41	35		.		2	45
		208	101	42	63	31	1		1	.	27
		209	12	13	9	11		.			53
	Totals		526	317	432	353	10	0	26	11	41
BC$_2$	204	210	16	50	24	60	1		1	3	73
		211	99	88	82	104		1	4	3	51
		212	112	62	114	91	5	5	4	8	41
	207	213	48	18	65	37	1	4		1	34
	200	214	91	71	47	106	2	5	6	1	56
		215	119	93	54	151	3	9	2	2	59
		216	158	89	87	98	3	3			43
		217	161	66	119	138	2	1	3	5	42
		218	80	85	128	129			1		51
		219	80	60	77	86	2	2	1		48
	206	220	179	50	181	55	2	3	6		23
		221	85	64	64	103	1	1	1		53
	209	222	57	47	34	50	1				51
		223	75	79	38	105	1	3	3	13	63
		224	121	61	62	108	2		1	2	48
	Totals		1481	983	1176	1421	26	37	33	38	48
BC$_3$	211	225	79	21	48	62	3		1		39
		226	130	39	84	109	10	4	4	1	40
		227	73	64	76	85		7	2	2	51
	211	228	69	50	53	57	1	3	2		47
		229	102	54	96	68		8	2	1	40
		230	86	30	77	37		2			30
	213	231	82	31	46	61		1	3	3	42
		232	68	38	55	83		2	1		50
	220	233	68	26	70	52	2		3	1	36
		234	74	58	87	66	9	2	1	4	43
	215	236	44	12	31	20		1			28
		237	57	60	34	89	6	1	1	4	61
		238	17	30	9	31	1	1	.	.	70
	Totals		949	513	766	820	32	32	20	16	44
	Grand totals		2956	1813	2374	2594	68	69	79	65	45

TABLE 10

P_1, *bar* ♀ × *tumor* ♂; *backcross*, F_1B ♀ × *tu* ♂.

CULTURE NUMBER	+	*tu*	*B*	*tu B*	PERCENT *tu*
249	142	30	138	20	15
250	117	36	197	44	20
251	343	47	311	47	13
252	182	19	165	24	11
253	262	18	200	14	6
254	270	55	249	40	15
255	116	29	115	31	21
Totals	1432	234	1375	220	14

cross generations showed no new relation. For the whole data the black-purple recombination percent was 2.8, instead of the standard 6. A similar reduction, to about 3, had been met with in other experiments, and was due to a specific crossing over reducer. The presence of a crossing over reducer made little difference, for the only conclusion that could be drawn from the data was that the locus of the principal second-chromosome tumor gene was probably rather near the right end of the chromosome.

BAR × TUMOR

The star dichaete × tumor backcross had shown that roughly a fifth of the flies of a tumor stock were made tumored by the added presence of genes outside the second and third chromosomes. The test for the presence in various parts of the X, of tumor genes of such slight effect, would be difficult. Only one experiment seemed simple enough to be worth a trial. The dominant mutant bar, with a locus at 57, might show non-random recombination with tumor, provided the locus of the tumor gene was rather close to that of bar. The bar × tumor backcross (table 10) showed no difference in the distribution of tumors to the bar and to the not-bar flies, 14 percent of the bar flies bore tumors and likewise 14 percent of the not-bars were tumor-bearing. Either the genes responsible for the above-mentioned margin were in the fourth chromosome rather than the X, or they were rather far to the left of bar in the X.

EYELESS × TUMOR

To test for the presence of fourth-chromosome tumor genes a tumor-bearing male was crossed to the fourth-chromosome recessive eyeless; and several cultures of F_2 were raised (table 11). Only 2 percent of the F_2 flies were tumor-bearing. The percentage of tumors in the eyeless

TABLE 11

P_1, *eyeless* ♀ ×*tumor* ♂; F_2, F_1+ ♀ ×F_1+ ♂.

CULTURE NUMBERS	+	*tu*	*ey*	*ey tu*	PERCENT *tu*
256	450	12	81	1	2
257	329	12	59	4	4
258	333	7	57	1	2
259	222	1	60	..	..
260	204	4	37	1	2
261	535	5	69	1	1
262	351	4	58	..	1
263	495	8	78	..	1
264	308	9	59	..	2
Totals	3227	63	558	8	2

TABLE 12

P_1, *tumor* ♀ ×*eyeless*, ♂; *backcross*, $t_u\ e_y$ ♀ ×F_1+ ♂

CULTURE NUMBERS	+	*tu*	*ey*	*ey tu*	PERCENT *tu*
265	136	41	77	13	20
266	112	36	93	30	24
267	175	48	133	25	19
268	202	37	100	15	15
269	142	23	47	5	13
270	35	13	23	16	33
271	169	31	101	18	15
Totals	971	229	671	122	18

was slightly, but not significantly, less than in the not-eyeless flies. Some of the eyeless tumor flies were inbred, and about 45 percent of the offspring bore tumors. A P_1 mating of eyeless to tumor gave F_1 males and these were backcrossed to eyeless tumor females of the above stock (table 12). About 18 percent of the backcross flies bore tumors, and of these the percentage of eyeless (15) was lower than the percentage in the not-eyeless (19). This difference was not great, but was as great as would be expected if there were present a fourth-chromosome modifier of the degree of effectiveness indicated by the results of the previous crosses.

SUMMARY

The development of the tumors studied was found to depend upon the action of differential genes that were numerous and that differed in effectiveness. The gene having greatest effect was a third-chromosome recessive located about 25 units to the right of roughoid and 15 to the left of dichaete. The second most effective gene was located in the second chromosome in the neighborhood of speck at the right end. About 80 percent of the total effect was due to genes in second and third chromosomes, and 20 percent to genes in the first and fourth.

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MATURATION IN DIPLOID AND TRIPLOID TOMATOES[1]

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INTRODUCTION

In the past few years numerous observations have been made which seem to indicate that abnormalities in chromosome distribution are less uncommon than was formerly thought. Triploidy appears to be one of the more frequent types, having been described for mosses, Morus, Canna, Datura, Hyacinthus, Uvularia, Oenothera, and Drosophila. Up to the present time no complete study of the early meiotic prophase of a triploid has been published although several authors have reported on the chromosome distribution and a few have studied the forms of the trisomes at diakinesis. It was thought that such an investigation might be useful both as a means of helping in the clarification of the vexed question of parasynapsis *versus* telosynapsis, and of determining the extent to which crossing over may be expected between members of a trisomic set of chromosomes in the tomato.

MATERIAL AND METHOD

Roots, anthers and ovaries from a triploid plant of *Solanum lycopersicum* L. were fixed in chrom-acetic-urea and stained with Heidenhain's iron haematoxylin. Anthers were also fixed in Flemming's strong. A short description of the plant and its origin has already been published (LESLEY and MANN 1925). Chromosome counts from pollen mother cells were made from smears stained by Belling's aceto-carmine method. Sections of whole buds were made to determine the time of maturation in the ovules in relation to that in the pollen mother cells. It was found in general that ovaries contained the desired stages when the pollen mother cells were in cytokinesis or immediately thereafter. Sometimes different anthers of the same flower were in very different stages and many degenerating anthers were found in both diploid and triploid buds. The genetics of the triploid plant is being investigated by J. W. LESLEY and it is to him that I owe the privilege of working on this material.

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[1] Contribution from the Genetics Division of the UNIVERSITY OF CALIFORNIA.

MATURATION IN THE DIPLOID

Seriation of stages

The maturation processes in the tomato were first studied from megaspore mother cells but since ovarian material does not give any adequate clue to seriation of certain critical stages, longitudinal sections of anthers were used for comparison. As long ago as 1904 BERGHS stated that neighboring cells in the anther sac of *Lilium lancifolium* often show a graded series of stages which give one a clue to the natural seriation in meiosis. JANSSENS (1905) speaking of the natural seriation shown by the testis of Batrachoceps says (p. 380)

> "We find ourselves then in the presence of an organ, which from the point of view of seriation of stages, offers the same advantage as the locules of the anthers of the Liliaceae."

No one anther provides a complete series in tomato, but a study of numerous anthers gives one an overlapping set of series from which most of the desired facts may be ascertained. The youngest stages are found at the proximal end; the oldest may be either at the distal end or near the center of the locule, usually the former. While the two locules of one anther contain approximately the same stages of meiosis, one may be slightly in advance of the other. The stages in the different anthers have been arranged in their natural order of progression from presynapsis to cytokinesis and are given below:

(1) Presynapsis (usually occupies whole locule)
(2) Leptotene to synezesis
(3) Synezesis to pachytene
(4) Synezesis (few cells at base of anther) pachytene to first anaphase
(5) Diakinesis to first telophase or sometimes second anaphase

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CRIPPLED, A NEW MUTANT CHARACTER OF *DROSOPHILA MELANOGASTER*, AND ITS INHERITANCE

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INTRODUCTION

This work was done in the Zoology Department of COLUMBIA UNIVERSITY from September 1923 to June 1924 and in the MARINE BIOLOGICAL LABORATORY, Woods Hole, from June to September 1924. I am very grateful for the hospitality shown to me by the authorities of these institutions. Also I am much indebted to Professor MORGAN, Doctor BRIDGES and Doctor STURTEVANT for valuable suggestions and advice.

ORIGIN OF CRIPPLED

The new mutant character, crippled, of *Drosophila melanogaster* was originally found in a black purple cinnabar fly. A female fly from the black purple cinnabar stock of the COLUMBIA laboratory was crossed with a male from the Swedish wild stock which had been kept breeding in the same laboratory. One of the F_1 wild-type females was backcrossed with a male of the former stock, and a wild-type female of the offspring was again backcrossed with one of her black purple cinnabar brothers. One black purple cinnabar female from the backcross was found to have her left hind tarsus curved sharply forward. This female was the ancestor of all the "crippled" flies which were found in the subsequent experiments and showed abnormality in the hind or middle leg.

Later some flies with the same kind of abnormality were found in the original black purple cinnabar stock. These gave by breeding some crippled offspring which showed no difference in the genetical behavior from the other crippled flies, so that it is very likely that this new mutant had arisen in that stock some time before it was first found.

TWO TYPES OF CRIPPLED, "CRIP-H" AND "CRIP-M"

This mutant character is a rather remarkable one because of its high variability. The middle or hind leg becomes abnormal if the fly has this factor in the homozygous state. When the character appears in the hind leg, some of the segments of this leg are shortened, or broadened, or crooked, or twisted, etc., but when the middle leg is affected by the presence of apparently the same factor, it forks or dwindles, or entirely disappears. And evidently the state of the food of the larva determines whether the hind or middle leg is to become abnormal. In the following descriptions this character will be called, for the sake of convenience, "crip-h" or "crip-m" according as it appears in the hind or middle leg, and simply "crippled," when there is no need of discrimination.

CRIP-H

Description

Crip-h is a highly variable character. The fly homozygous for its factor may be quite normal somatically, but often has one of the hind legs more or less abnormal. Any of the segments of the leg may be shortened, broadened, straightened, crooked or twisted. Sometimes the abnormality is restricted to a single segment, but at other times more than one segment are deformed. Also it is quite frequent that the leg lacks some of the distal segments. In the extreme cases the entire leg is missing, leaving no trace at all. Figure 1 shows some of such crip-h legs.

Except in the cases where the abnormality is very slight, the crippled leg is apparently no longer functional; the fly drags it while walking. Moreover, such a fly often has one wing folded, owing to the hind leg of the same side being crippled and incapable of extending it.

As mentioned above, this kind of abnormality occurs as a rule in only one of the hind legs. But rarely both of the legs become abnormal at the same time; in these cases there is no similarity in the grade or type of the abnormality of the two legs. Exceptionally the middle leg also shows the same kind of abnormality; in these cases the hind leg of the same side is also abnormal, practically without exception, and the abnormality is usually greater in degree in this leg than in the middle leg. Such cases of the abnormality of the middle leg belong to a category different from crip-m, as will be mentioned later.

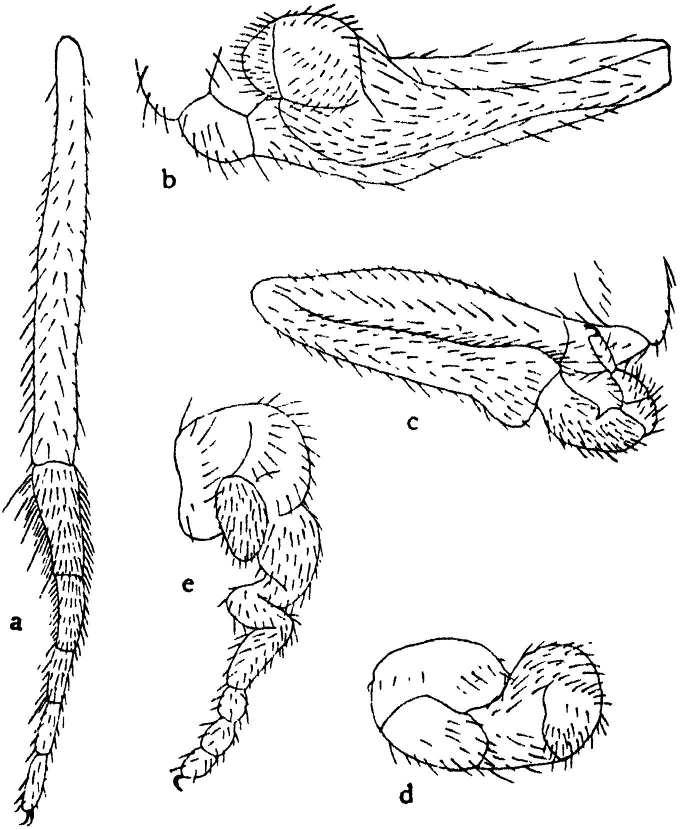

FIGURE 1.—Some forms exhibited by crip-h legs.

(a) Tibia straightened; first and second tarsal segments slightly broadened and curved. (b) Femur somewhat deformed; tibia swollen considerably at distal end; tarsal segments fused into spoon-shaped body, terminal claws missing. (c) Femur and tibia somewhat deformed; tarsus curved sharply forward and upward, with segments, especially the proximal two, swollen and deformed. (d) Femur crooked; tibia and tarsus missing. (e) Entire leg shaped something like a cork-screw; femur much shortened; tibia and proximal part of tarsus twisted. ×70.

Influence of environment

Age of parents

Before making the experiments on the effect of environment upon the character crip-h, special attention was given to whether the age of parents had any effect on that character in the offspring. Thus, a pair of flies homozygous for the factor were left to lay eggs in the first bottle for eight days, and before the flies of the next generation began to emerge, they were transferred to a second bottle and left for the next eight days. Sometimes a third bottle was prepared in the same way. These two (or three) bottles were kept in the same incubator and the flies that emerged from them were counted and the percentages of the crip-h flies of the different bottles were compared. In all a dozen sets of bottles were prepared. Thus, I found that in some cases a relatively greater number of crip-h flies came out of the bottles of the later broods than from those of the earlier, but in other cases the reverse was true, and in still other cases the result was self-contradictory, that is, in one class there was a greater number of abnormal flies among the earlier broods than among the later, whereas in another class more crip-h flies were found among the later broods than among the earlier (classification being made in reference to some characters other than crip-h, for example, black, purple etc.). In short, the age of parents did not seem to play any definite rôle on the appearance of the crip-h character in the offspring.

High temperature

Of several pairs of bottles, prepared in the same way as described above, the first bottles were put into an incubator at a temperature 29° to 30° C, right after the removal of the parents, while the second bottles were kept in the 25°C incubator throughout. The percentages of the crip-h flies in the two types of bottles were compared. Sometimes the second brood was reared in the high-temperature incubator, while the first one was left in the 25°C incubator. The high temperature shortens the duration of the larval and pupal period more or less, sometimes by two days, so that the counting of the flies was given up on the eighth day after the first fly had appeared in the bottle. Thus, I found that, in seven out of eight sets of bottles there were relatively more crip-h flies in the high-temperature bottles than in the 25°C bottles, the difference amounting to 35 to 80 percent of the percentage of the crip-h flies in the 25°C bottle in most of the sets. In only one set the 25°C bottle yielded more crip-h flies than the corresponding high-temperature bottle. But in this set the brood of the former bottle was much smaller than that of the latter, and, as will be shown later, the percentage of the crip-h flies depends much on the size of the brood, the percentage being smaller the larger the brood, so that this case cannot be taken as contradictory to the general result.

Low temperature

A similar experiment was carried out for low temperature. The ordinary ice-box was used to get low temperature, which fluctuated from 8°C to 15°C. Such temperatures retarded the development of the larvae considerably, and the F_1 flies were counted for about a month after the first one had appeared. Most of the cultures produced less than one hundred flies during this time.

Of each set, consisting of the two bottles containing two successive broods, usually the first one, but sometimes the second one, was put in the ice-box. In all, ten sets of bottles were prepared.

The bottles kept in the ice-box produced relatively much larger numbers of crip-h flies than the corresponding bottles that had been kept in the 25° C incubator. The percentage of crip-h flies in the former was in most cases 65 to 75, whereas in the latter it was usually 20 to 30. It is clear then that, even if due allowance is made for the difference in the size of the broods of the cold and ordinary bottles, the difference in the percentages of crip-h flies was fairly great.

Three F_2 cultures from flies heterozygous for the factor for crippled were reared in the same ice box. From these cultures there appeared 17.7 to 24 percent crip-h flies, in all of which the abnormality was rather slight. A brood of black jaunty flies having no factor for crip-h, reared in the same ice-box, produced no crip-h flies among the progeny.

State of food

There is a more or less remarkable difference in the percentage of crip-h flies in the earlier and later counts of one and the same bottle. This difference is as a rule the more apparent the larger the brood. When the brood consists of as many as 300 to 600 flies, the percentage of crip-h flies in the last five days is often less than half that for the first five days. When the brood is smaller, this difference is less marked, and the percentage of crip-h flies in the bottle, taken as a whole, is higher than that of the other bottles containing larger broods, In the cultures in which more than one pair of crip-h flies were allowed to lay eggs, or in which the food was less than usual, this difference was especially marked. These facts show that this change in the percentage of the crip-h flies was closely associated with the change in the state of food induced by the larvae feeding on it. But it is not clear just what kind of chemical or physical change in the food is responsible for this change in the flies. This question will be taken up again later.

Inheritance

When a crip-h fly is crossed with a fly of an unrelated stock, most of the F_1 offspring are normal and very few are abnormal. The percentage of the abnormal flies is almost always lower than 1, sometimes being 0; in other words, this character shows recessiveness in the majority of cases, but a slight dominance in some exceptional cases. In F_2 appear numerous abnormal flies which are homozygous for the crip-h factor. The number of abnormal flies, however, is variable and always smaller than expected on the basis of a simple recessive factor, since flies that should be crippled, genetically, may be quite normal somatically, and one can tell only by breeding tests that they are homozygous for that factor.

The cross between the two flies homozygous for the crip-h factor gives usually 10 to 30 percent crip-h flies, more rarely 30 to 40 percent, the percentage practically never amounting to 50.

Evidently no special type of the abnormality is inherited. Not only do the crip-h flies of various types emerge from one and the same bottle, but a somatically normal pair of parents may produce a number of very abnormal flies, just as do somatically very abnormal pairs. Also, it seems to be determined at random which (right or left) of the hind legs is to become abnormal.

Locus of the factor for crip-h

Some black purple cinnabar crip-h males and females were crossed with star dichaete flies, and several of the F_1 star dichaete males were backcrossed to black purple cinnabar crip-h females. None of the crip-h flies that appeared in the next generation was star, except a few star crip-h flies which were tested, and found heterozygous for the crip-h factor. It is clear, then, that the factor for crip-h lies in the second chromosome. This fact was also proved by mating the black purple cinnabar crip-h fly with the wild fly and then backcrossing the F_1 fly with the black purple cinnabar crip-h fly. When the F_1 male was backcrossed with the black purple cinnabar crip-h female, all the crip-h flies that appeared in the next generation were also black purple cinnabar. When, on the other hand, the F_1 female was backcrossed, most of the crip-h flies of the next generation were also black purple cinnabar, but there appeared a few flies that were purple cinnabar (not-black) and crip-h.

The fact that the purple cinnabar (not-black) fly may be crippled, suggests that the factor for crip-h is located to the "right" of black. Accordingly, ten black (not-purple not-cinnabar) flies and sixteen purple cinnabar (not-black) flies which had come from crossing over between the loci, black and purple, were tested for the crip-h factor. It was found that black (not-purple not-cinnabar) flies were never homozygous for the factor in question, while purple cinnabar (not-black) flies were homozygous for it without exception. These experiments have shown that the factor for crip-h probably does not lie between the loci black and purple, but is located between the loci purple and cinnabar. Next, six black purple (not-cinnabar) flies and seven cinnabar (not-black purple) flies which had come from the crossing over between the loci purple and cinnabar were tested. Five of the six black purple flies and five of the seven cinnabar flies were found to be homozygous for the crip-h factor, proving that the factor lies between the loci purple and cinnabar, which are known to be at about 54.5 and 57.5, respectively (BRIDGES and MORGAN 1919; CLAUSEN 1924; and MORGAN, BRIDGES and STURTEVANT 1925).

CRIP-M

Origin and description

In the course of the experiments described above, a somewhat different kind of crippled fly began to appear in some of the cultures. Such flies were first found in a certain strain, but appeared later also in a few other strains somewhat remotely related to it, and also to one another. In this particular kind of crippled the leg that becomes abnormal is one of the middle pair instead of the hind legs. Moreover, the abnormality belongs to a category different from the abnormality of the hind leg. The case most commonly met with has the leg entirely missing. More rarely it is represented only the coxa, or by the coxa and trochanter with or without a small vestige of some of the more distal segments (figure 2, g). Sometimes the leg is smaller in size than the normal leg, but is quite normal in shape and in proportional size of segments. In the majority of cases only one of the middle legs is abnormal, but rarely cases are found in which both of them are missing and the fly is four-legged.

Crip-m is a character sharply distinguishable from crip-h, and few cases have been met with in which the discrimination is hard. As mentioned above, crip-h flies may sometimes have the middle leg of the same side also somewhat abnormal. In these cases the middle leg is usually less abnormal than the hind leg. In crip-m, on the other hand, only the middle leg becomes abnormal, the hind leg remaining quite normal. Moreover, these two types of the crippled fly are distinguishable from each other by the time of appearance; crip-m flies appear only after the culture has become old, mostly during the last few days of counting when relatively few crip-h flies are appearing.

The number of the crip-m flies that appear from one culture is very small; it very rarely amounts to 5 to 8 percent of the total number of flies of the bottle.

Doubling of the middle leg

In crip-m flies the pleura of the thorax also often shows abnormality in some way or other. In the flies that lack one of the middle legs a depression is usually found at the part of the thorax where the leg should be. This depression may be so large as to cover the base of the sternopleural bristles, which are then also missing. In the flies with the middle leg smaller than normal, on the other hand, the pleura often has a sign of doubling as indicated by another set of the sternopleural bristles situated above the ordinary set. In such flies the part between the two sets of bristles commonly carries a rudiment of the leg. This rudiment is, as a rule, very small and imperfect, consisting of only one or a few segments (figure 2, a), but rarely it is fairly large and quite normal in proportion. There are even cases in which two practically perfect middle legs occur on the same side of the body (figure 2, b). There are also cases in which not the whole leg, but a part of it, is reduplicated, as shown in figure 2, c to f.

Such cases of the doubling of the leg remind one of the character "reduplicated" described by Miss HOGE (1915). "Reduplicated," however, was a sex-linked character and appeared in any leg, but predominantly in the fore leg, whereas the character before us is not sex-linked and appears exclusively in the middle leg. Moreover, the doubling seen here belongs to a category different not only from "reduplicated" but also from most of the cases of reduplication of the leg described in insects before. This fact may be clear from the following "rules" which have been formulated from observations on more than thirty cases of the doubling of the leg.

Rules of doubling

(1) The doubling may occur at any level of the leg.

(2) The parts contained in the branch are only those that are distal to the point of division.

(3) In all cases of doubling, one branch or one leg is in the normal position and direction.

(4) The plane of division of the leg into two primary branches may coincide with the vertical (dorso-ventral) plane or with the horizontal (antero-posterior) plane of the leg.

(5) When the division is by the vertical plane, the resulting two branches are mirror images of each other, the anterior surfaces facing.

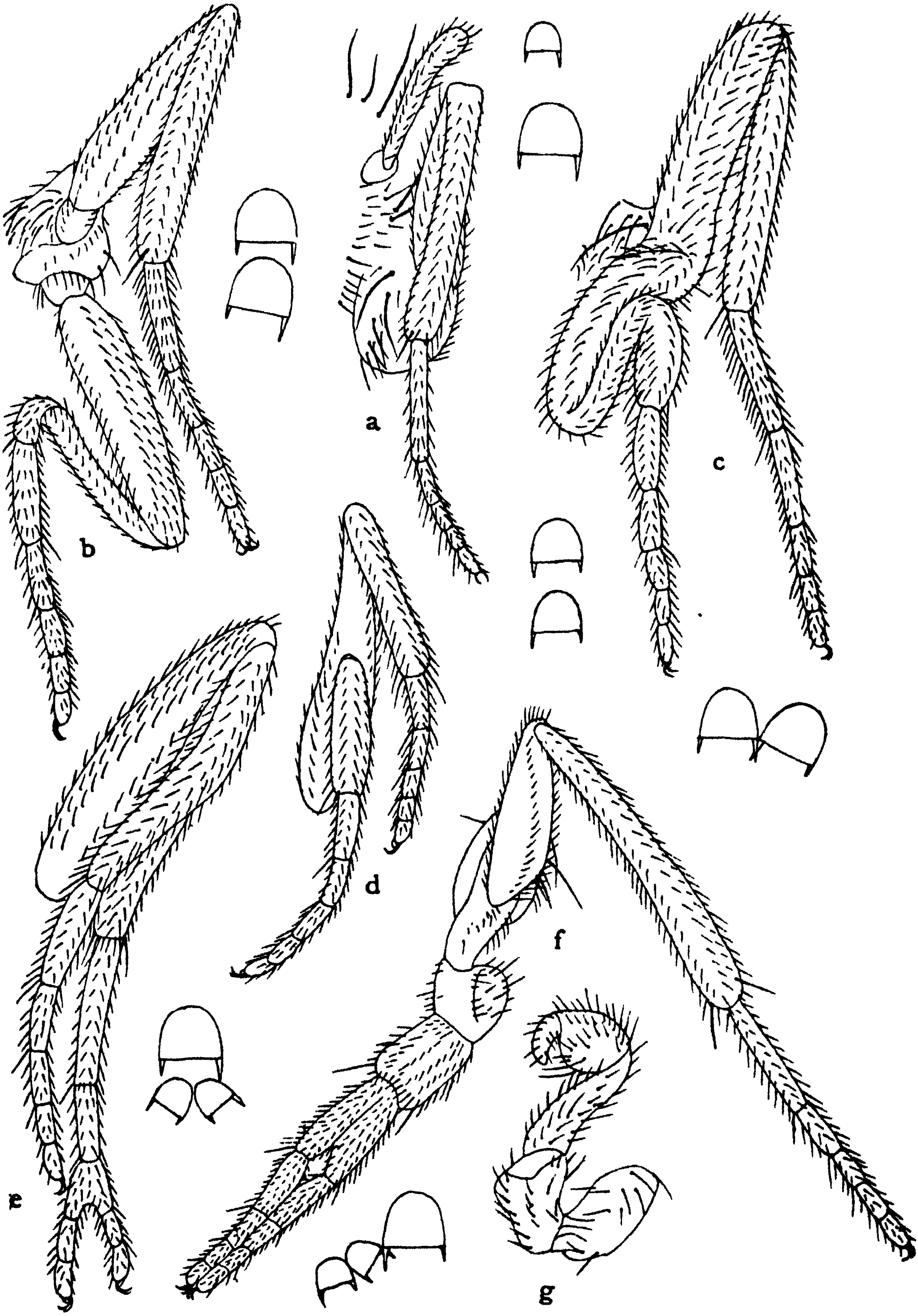
a
b
c
d
e
f
g

FIGURE 2. Median legs of crip-m flies. The accompanying diagrams show the side and direction of branches. All ×65.

(a) A small rudimentary median leg.

(b) Two nearly perfect median legs on the same side of the thorax.

(c) Left leg. Femur divided at base by a vertical (dorsi-ventral) plane; both branches complete, of about normal size, facing each other with anterior surface; supernumerary branch curved sharply near middle of tibia and folded up.

(d) Right leg. Limb, as large as the original limb and consisting of tibial and tarsal segments, attached on the ventral surface of the femur at about the middle of it; both limbs being right limbs.

(e) Left leg. Femur compound, that is, consisting of two branches fused into one, much thicker than normal; tibia bifurcated at base by horizontal (antero-posterior) plane, forming two branches, one overhanging the other; dorsal branch with tibia curved slightly; ventral branch with tibia, first and second tarsal segment compound, third divided by dorsi-ventral plane.

(f) Right leg. Trochanter divided by vertical plane; posterior branch normal in position and in proportion of parts; anterior branch with femur much shortened and deformed, tibia short and thick, compound; tarsus divided at first segment into two secondary branches with dorsal surfaces facing together.

(6) When the division is by the horizontal plane, the resulting two branches are of the same side (not mirror images of each other) and one hangs over the other.

(7) Of the two primary branches, the one in the normal position never undergoes further division, while the other may do so.

(8) When the secondary division occurs in the primary branch, the plane of division is usually vertical to the plane of the primary divisions,—if the primary plane is dorso-ventral, the secondary plane is horizontal, and *vice versa.*

(9) The two secondary branches are mirror images of each other.

The division of the leg by the horizontal plane into two branches, one overhanging the other, is very characteristic of the present case. As far as I know, few instances of this category have ever been described (Bateson, 1894, 1913). It is not uncommon here; in fact, I have observed more than a dozen cases belonging to this category.

Influence of state of food on crip-m

The crip-m flies appear almost without exception during the latter half of the counting, that is, from about the fifth day after the appearance of the first fly in the culture. This time coincides with the time when the percentage of the crip-h flies begins to lower. In a culture that throws relatively many crip-m flies the percentage of crip-h flies is comparatively low from the beginning. And if one keeps such a culture rather long, —more than one month—one commonly finds that the crippled flies that appear from it are mostly crip-m. It is thus very likely that one and the same cause is acting here both for the decrease of the percentage of the crip-h flies and for the production of the crip-m flies. And this cause is evidently environmental and connected with the change in the nature of the food, as shown by the results of the following two experiments:

First, two successive broods of crip-m flies were raised from the same parents; in all six such sets were prepared. In every set the two broods showed practically the same change in the type of the crippled flies from crip-h to crip-m.

In the second experiment, a pair of black purple cinnabar crip-m flies were introduced into a bottle, from which only black purple flies had been appearing for more than ten days and in which most of the crippled flies were then crip-m. Two out of ten such bottles produced offspring from the introduced pairs. The crippled flies among those offspring were mostly crip-m besides being black purple cinnabar.

The banana culture-medium is strongly acid at the start of the culture, then decreases its acidity gradually as many flies come out, and becomes weakly alkaline towards the end of the counting. I thought it might be this change in the chemical nature of the food that is mainly responsible for the change in the type of the abnormality of the fly. I therefore neutralized to a certain extent some new acid cultures with sodium bicarbonate or with ammonia water and also acidified some old alkaline cultures with acetic acid, but there was no recongizable effect on the percentage of the crip-h or crip-m flies that came out subsequently in either experiment. Some cultures were also kept extremely moist from beginning to end by adding water from time to time, but with no apparent effect. Thus, although it is likely that the change in question is induced by some change in the nature of the food, yet my experiments have failed to reveal just what kind of chemical or physical change is responsible for it.

Inheritance of crip-m

When crip-m is crossed with flies of an unrelated strain, the F_1 flies are all normal. When two crip-m flies are mated, there appear a number of crip-h offspring during the first half of the counting; then the number of the crip-h flies decreases; and, toward the end of the counting, a few crip-m flies come out. The result is just the same when two crip-h flies of the same strain are crossed together or a crip-m fly is mated with a crip-h fly.

To find the locus of the factor for crip-m in the chromosome, a few black purple cinnabar crip-m flies were mated with wild flies, and four F_1 females were backcrossed with black purple cinnabar crip-m males. From each bottle there appeared a few crip-m flies besides a number of crip-h flies. All of the crip-m flies were black purple cinnabar, except one black purple (not-cinnabar) and one cinnabar (not-black not-purple) crip-m fly. This result shows that the factor for crip-m character lies in the second chromosome somewhere between purple and cinnabar. Next, five crip-m females were mated, each with a male from the original black purple cinnabar stock bottle. As mentioned before, the flies from this stock may have carried the factor crip-h, but there is no reason to suspect that the factor for crip-m, if any special factor for this character exists, has been present in them. These five females all gave a few crip-m offspring besides a number of crip-h flies, showing that the factor for crip-m is probably the same as that which produces crip-h.

It is not known why no crip-m flies had been found at the beginning of this whole experiment on cripplied, but appeared later in some particular cultures. Possibly we have to deal with a modifying factor here. However, the number of crip-m flies that appear in each culture is too small to enable us to decide this question easily.

It might be suspected that the absence of the middle leg and the reduplication of the same would possibly represent entirely different characters. But this is evidently not the case. In fact, I often mated two flies having a reduplicated middle leg together, and also such a fly with a fly lacking a middle leg; these crosses always gave practically the same results as the cross between two flies lacking the middle leg. Moreover, there were series of types of abnormality of the middle leg from the doubling of the leg to its complete absence.

SUMMARY

1. Crippled is a new mutant character which appears in one of the middle or hind legs of *Drosophila melanogaster*. When it appears in the hind leg (crip-h), some segment or segments of this leg are shortened, or broadened, or crooked, or twisted or entirely missing. When it appears in the middle leg (crip-m), this leg forks, or dwindles, or entirely disappears.

2. Crippled is a recessive character. The cross between two flies homozygous for the factor gives usually 10 to 30 percent crippled offspring, most of which are crip-h and a few may be crip-m.

3. The percentage of the crip-h flies is higher in broods reared in high temperature (29° to 30°C) or low temperature (8° to 15°C) than in those bred in the optimum temperature (25° C).

4. The percentage of the crip-h flies that appear from the same bottle is higher during the first five days than during the last five days of the counting.

5. The factor for crip-h lies in the second chromosome between the loci purple and cinnabar.

6. The crip-m flies appear only near the end of the counting when the percentage of the crip-h flies is comparatively low.

7. The decrease of the percentage of the crip-h flies and the production of crip-m flies near the end of the counting, seem to be due to one and the same cause, namely, a certain state of the culture medium; but several attempts to identify the cause were unsuccessful.

8. When the reduplication of the middle leg occurs, the leg may be divided either by the vertical or by the horizontal plane. When it is divided by the vertical plane, the resulting branches are mirror images

of each other; when it is divided by the horizontal plane, the branches are of the same side, not mirror images.

9. One and the same factor probably produces both crip-h and crip-m.

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ON THE OCCURRENCE OF MUTATIONS WITHIN TRANSPLANTABLE NEOPLASMS[1]

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INTRODUCTION

In a survey of the recent work on the inheritance of susceptibility to a transplantable tumor, two general conclusions of importance can be deduced. The first of these conclusions formulated by LITTLE and STRONG (1924) is that the fate of the implanted tumor tissue is determined by a reaction between the host and the transplantable tumor cells. The reaction of the host is governed largely by its genetic constitution (derived from the zygote from which it arose) and that of the transplantable tumor is somewhat controlled by certain intrinsic or genetic factors. The expression "reaction potential" perhaps defines this play of factors which determine the "transplantability" of a tumor more accurately than the older expressions "proliferative or growth vigor" and "adaptability." These older expressions overemphasize the rôle of the tumor transplant at the expense of the important part played by the host.

The second conclusion, concerning the host's tolerance of a transplantable tissue is more specific, "susceptibility to a transplantable neoplastic tissue is brought about by the simultaneous presence of *multiple* mendelian (genetic) factors, introduced into the individual at the time of the fertilization of the egg" (LITTLE and STRONG 1924). With the use of the term "simultaneous presence," we do not intend to imply any reference to the discarded theory of presence and absence first introduced by Bateson. We use the term "presence" for purely descriptive purposes.

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[1] The experiment here reported has been made possible by a grant from the Rockefeller Institute for Medical Research.

For example, if susceptibility to a transplantable tumor depended upon the simultaneous presence of two genetic factors, *A* and *B*, (as in the case of the adenocarcinoma, *dBrB*), then the mice with the following genetic constitutions would be susceptible; *AABB*, *AaBB*, *AABb* and *AaBb*. The mice with the following genetic constitutions would be resistant to the transplant; *AAbb*, *Aabb*, *aaBb aaBB* and *aabb*.

The Mendelian expectations for susceptible and non-susceptible individuals, involving the several possible combinations of "simultaneous factors," for the two generations, the F_2 and the backcross to the negative strain, are given in table 1.

TABLE 1

Expected proportions of susceptible (+) and non-susceptible (−) individuals in the two important generations, the F_2 and the backcross to the negative strain, on the assumption that for the continued growth of the transplant there must be present one or more definite genetic factors within the host.

NUMBER OF FACTORS	FACTORIAL COMPOSITION OF F_1	EXPECTATIONS F_2	RATIO IN F_2	PERCENT NEGATIVES F_2	EXPECTATIONS, BACKCROSS
1	*Aa*	3+ : 1−	1+ : 0 33−	25.00	1+ : 1−
2	*AaBb*	9+ : 7−	1+ · 0.78−	43.75	1+ : 3−
3	*AaBbCc*	27+ : 37−	1+ : 1 37−	57.81	1+ . 7−
4	*AaBbCcDd*	81+ : 175−	1+ : 2 16−	68 38	1+ : 15−
5	*AaBbCcDdEe*	243+ : 781−	1+ : 3.21−	76.27	1+ : 31−
6	*AaBbCcDdEeFf*	729+ : 3367−	1+ : 4.61−	82.20	1+ : 63−
7	*AaBbCcDdEeFfGg*	2187+ : 14197−	1+ : 6 49−	86.65	1+ : 127−
8	*AaBbCcDdEeFfGgHh*	6561+ : 65536−	1+ · 9 98−	90.89	1+ : 255−

EXPERIMENTAL METHODS

The tumor which is the subject of this investigation is the third spontaneous neoplasm to arise in a relatively homogeneous dilute brown strain of mice which had been rigidly inbred, brother to sister, for at least forty generations. This tumor, which has not been previously described, we have designated as *dBrC*, in order to continue the nomenclature used for the other tumors arising in this same stock which were called *dBrA* and *dBrB*. On transplantation it was found, as with the previous tumors, that all animals of this dilute brown strain were uniformly susceptible. On the other hand, the BAGG albino strain, another inbred stock, was found to be uniformly resistant to transplants of this tumor as it had been to tumors A and B of the same stock.

The F_1 generation produced by crossing these two stocks gave individuals all of which grew the transplant (180+ :0−). At the present time, 125 individuals from the F_2 generation have been inoculated with this transplantable tumor, *dBrC*, and 23 of these grew the transplant, while 102 proved to be resistant. In attempting to fit the observed data to mendelian principles, as has already been so successfully done in the case of four transplantable tumors (two by Tyzzer and Little and two by Little and Strong 1924), it was evident that the behavior of the transplant placed it in the same class as the two other transplantable tumors derived from this same inbred dilute brown stock of mice. The results can be explained if the assumption be made that for the successful growth of the transplant there must be present within the host at least from five to seven independently inherited genetic units or factors (genes). It is probable that the number of genetic units involved is six. The degree of probability determined by the comparison of the probable errors between observation and expectation for five, six and seven factors, is greater in the case of six factors than it is for the other two possibilities, although the five and the seven factor interpretation is not definitely excluded by the present data.

The degree of significance between the observation on the original *dBrC* tumor and the expectation according to Mendelian principles is given in table 2.

TABLE 2.

Comparison of the observed data for the F_2 generation with the expectation for five, six and seven Mendelian factors, in case of original dBrC tumor.

	F_1 GENERATION	NUMBER OF INDIVIDUALS	PERCENT NEGATIVES	DIFFERENCE BETWEEN
1	Observa-vation	23.00+ : 102− ±2.89	81.60±2.30	
2	Expecta-tion 5 Factors	29.69+ : 95.31− ±3.17	76.09±2.69	1 and 2=5.51%±3.53 or 1.56×PE
3	Expecta-tion 6 Factors	22.28+ : 102.72− ±2 85	82.16±2.30	1 and 3=0.56%±3.25 or 0.17×PE
4	Expecta-tion 7 Factors	16.69+ : 108.31− ±2.54	86.65±2.03	1 and 4=5.05%±3.05 or 1.65×PE

After the collection of the above data, no further genetic studies being contemplated, the tumor was simply continued for some generations in the original dilute brown stock. In the course of this routine continuation of the tumor it was found that one of the transplants grew with remarkable rapidity. In sub-transplants from this particular tumor the rapidity of growth continued. Whereas the original *dBrC* tumor, growing in the susceptible homogeneous dilute brown mice, would rarely attain a weight of a gram and a half to two grams in from two to three months, this single mass has produced upon further transplantation growths which have reached from twenty-two to twenty-three grams in from four to five weeks. Obviously there must have been a clear cut variation from the type reaction which had been investigated for over a year. Consequently this new tumor was called *dBrCX*, the *X* being attached to the original formula signifying some unknown characteristic of the new transplantable tumor. On further study it soon became apparent that the substrain had developed new tissue characters. *dBrCX* grew in all F_2's, in all F_1's, and in all individuals belonging to the original dilute brown mice, in all individuals belonging to several backcross generations toward the original non-susceptible stock, in all individuals belonging to the original non-susceptible stock, and in fact in all mice irrespective of their genetic relationships. It grows equally well in wild mice and mice belonging to a stock of mice imported from England several years ago and kept entirely distinct by brother-to-sister matings since their importation. From a state of high specificity, the transplantable tumor had become completely non-specific. Correlated with this loss of tissue specificity there was also obtained an increased growth or proliferative vigor. There was apparent a degree of significance of difference between the original *dBrC* and the new *dBrCX* tumor amounting to 32.57 times the probable error. From the time at which this distinct deviation from the normal growth rate of the transplantable tissue was observed we made careful observations with the possibility of detecting other deviations from the estimated normal growth rate. As a result of this procedure, two transplants were selected which seemed to show a difference. These have since received the names of *dBrCm* and *dBrCsp*. *dBrCm* will grow in a given host, provided there be apparent the simultaneous functioning of at least two independently segregating Mendelian units, that is, there is obtained a typical 9 : 7 ratio in the F_2 generation. The ratio of susceptible mice to non-susceptible mice in the F_2 generation for the *dBrCsp* tumor is probably 3 : 1. In other words, the physiological difference between susceptibility and non-susceptibility is apparently controlled by the functioning of one

TABLE 3

The data obtained for the three transplantable tumors, dBrCm, dBrCsp and dBrCX, in the F_2 generation, together with the comparable data for the original dBrC tumor.

NAME OF TUMOR		F_2 GENERATION	NUMBER OF INDIVIDUALS	PERCENT NEGATIVES	DIFFERENCE BETWEEN
Original *dBrC*	1	Observation	23 00+ : 102.00− ±2.89	81.60% ±2 30	
dBrCm	5	Observation	99.00+ : 65.00− ±4 17	39.64% ±2.53	1 and 5 = 41.96% ±3.42 or 12.26×PE.
	6	Expectation 1 Factor	123.00+ : 41.00− ±3.55	25 00% ±2.16	5 and 6 = 14.64% ±3.32 or 4.41×PE.
	7	Expectation 2 Factors	92.25+ : 71.75− ±4.23	43.75% ±2.57	5 and 7 = 4.11% ±3.60 or 1.14×PE.
	8	Expectation 3 Factors	69.20+ : 94.80− ±3.60	57 81% ±2.20	5 and 8 = 18.17% ±3.35 or 5.42×PE.
dBrCsp	9	Observation	75.00+ : 23.00− ±2.79	23.47% ±2.86	1 and 9 = 58.13% ±3.67 or 15.84×PE.
	10	Expectation 1 Factor	73.50+ : 24.50− ±2.85	25.00% ±2.90	9 and 10 = 1.53% ±4.07 or 0.37×PE.
	11	Expectation 2 Factors	55.10+ : 42.90− ±3.27	43.75% ±3.36	9 and 11 = 20.28% ±4.41 or 4.59×PE.
dBrCX	12	Observation	243.00+ : 2.00− ±0 94	0.82% ±0.38	1 and 12 = 80.78% ±2.48 or 32.57×PE.

Mendelian dominant factor introduced into the individual with the formation of the zygote. The data obtained for the three transplantable tumors, *dBrCm*, *dBrCsp* and *dBrCX* in the F_2 generation together with the comparative data for the original *dBrC* tumor are given in table 3.

RESULTS

A transplantable tumor, known as *dBrC*, gave very uniform results in a series of mice of known pedigree for a period of about a year. We may say that the reaction potential existing between the tumor and the individuals inoculated was the same during this first part of the experiment. This tumor will grow in a mouse progressively provided there be present simultaneously at least from five to seven independently segregating genetic factors (genes) in the fundamental make-up of the host derived from the zygote that gave rise to the individual. In the course of routine transplantation of this tumor, there was a sudden change in this hitherto constant reaction potential resulting in at least three types of transplantable tumors—new types as far as their physiological activity and reaction potential are concerned. With this change in the reaction potential of the transplantable tumor there is also involved or correlated with it a changed proliferative vigor and other physiological changes. From a single original transplantable mass, I have therefore obtained four masses that remain true to type at least for some months of continued experimentation. These four types are (1) the original tumor, *dBrC*, still giving a 5–7 mendelian factor ratio, (2) *dBrCm* giving a two factor ratio, (3) *dBrCsp* showing a one factor ratio, and (4) *dBrCX* growing in all mice inoculated—that is non-specific. The number of factors involved in the "transplantability" for the several tumors investigated is given in table 4.

TABLE 4

Tabulated synopsis of the various transplantable tumors used in the present experiment, showing the probable number of independently segregating genetic factors involved in the process of transplantation.

NAME OF TUMOR	NUMBER OF GENETIC FACTORS
Original *dBrC*	6
dBrCm	2
dBrCsp	1
dBrCX	Undetermined.

GENERAL DISCUSSION

Transplants from a spontaneous cancer rarely grow well in the first generations. Generally, however, the number of successful takes increases with subsequent transplantation till a very high rate is attained. There are two important theories formulated to explain this supposed increased ease of transplantation. The first theory may be called the virulence theory, although this term is rather unfortunate on account of certain implications from the study of infectious diseases. The second theory has been called the adaptation theory and is described by Woglam (1919) as "The presence (or absence) of power to adapt themselves to new hosts appears to be a deciding factor in the success (or failure) of most spontaneous mouse carcinomata after transplantation." The first theory is fostered by Ehrlich and Apolant, the second by Bashford, Murray, Haaland, Bowen, Cramer and more recently by Woglom.

The data contained in this paper have a direct bearing upon these two theories. It is very probable that neither the theory of adaptation nor the theory of virulence will explain completely the evidence outlined. These theories are descriptive phrases applied to the tumor mass and enter into the equation after the change or changes have been produced—that is, they are the result of the change and not the cause of the phenomenon. As in the case of so many other alternative hypotheses, a third explanation is sometimes called for that seems to fit the data better than either of the original hypotheses.

Before attempting to formulate this new hypothesis, let us enumerate a few of the features involved in the transplantation of malignant tumors, that have a bearing on the problem at hand. (1) The cell is the unit of structure in the malignant tissue as well as it is in the normal tissue. (2) The inoculation of a given mass consisting of malignant tissue results in a true transplantation of tissue and is not an infectious process. By this is meant that the cells making up the mass of the new growth are lineal descendants from the cells introduced into the host at the time of inoculation. (3) Very few cells placed in the new host give rise to the new growth; most of them die during the process. (4) Uniformity of behavior of the transplantable tumor implies, of course, that there must be a relatively stable mechanism for the perpetuation of this uniformity.

With these conceptions concerning the process of the transplantation of malignant tissue before us, we are now ready to attempt an analysis of this phenomenon of the change in the reaction potential encountered in the present experiment. The change, whatever it is, must of course be either in the host component or in a possible tumor variable.

Obviously, if the host's tolerance of a transplantable tumor is dependent to a large extent at least upon its genetic constitution, when a change occurs in the genetic constitution of the host then this change, of necessity, would alter the reaction potential under consideration. Changes in the genetic constitution of the mice, however, cannot explain the present data. The second filial generation individuals, which give the indication of the genetic complex involved so far as the host is concerned have been picked out at random and inoculated with one type of transplantable tumor or another. The parents were the same for all the F_2's, while the number of susceptible grandparents was very small. Sometimes a single mouse was inoculated with two different types of tumors simultaneously. The genetic constitution of the mouse, as far as this experiment on testing its physiological response to two distinct types of tumors is concerned could not possibly be different. For these two reasons, the host complex during this experiment must have been a constant one or, at least, has not appreciably influenced the observed change in the reaction potential. The variable factor has not been within the mice employed.

We are therefore led to the conclusion that the only other variable that may be functioning is the transplantable tissue. There are two alternative conceptions concerning the mechanism of how this change in the tumor may have been brought about. (1) The original transplantable tumor employed was a mixed tumor, or (2) There has been a change in the genetic constitution of the unit of structure of the transplantable mass.

For several very obvious reasons, that need not be entered into here, the original tumor could not have been a "mixed" tumor.

The other conception is that this change of the reaction potential existing between the host inoculated and the tumor is brought about by an internal change in the genetic constitution of the tumor cell. This conclusion of course implies that the tumor cell is controlled to some extent at least by an internal genetic constitution.

Changes within the genetic constitution of living forms, if they are germ cells are called mutations; if they are somatic cells (occurring especially in plant tissues) are called somatic mutations. The conclusion is therefore reached that somatic mutations may occur within the tumor cell, and that when these mutations do occur, they change the reaction potential and other physiological activities of the tumor cell. The data at hand are not sufficient to determine more in detail the nature of this mutational process. It may be either a change or shifting of a complete chromosome or chromosomes, or a change or changes within a chromosome or chromosomes (genic), or it may be even cytoplasmic in nature. By mutation, I merely mean to use the term in its broadest sense, that is, a change or shift within the genetic or internal constitution that results in definitely clear cut or discernible differences in behavior or structure that is perpetuated by the process of heredity (in this case, cell division).

We have therefore in the analysis of the data obtained in this experiment an explanation of the phenomenon of the so-called adaptiveness or virulence of the tumor cell. I have already pointed out the similarity between the data outlined in this paper and the data upon which the theories of adaptiveness and virulence or proliferative vigor are founded. If a genetic change such as a somatic mutation is responsible for the change in the reaction potential of the transplantable tumor cell and other physiological activities, such as the growth vigor then it is highly probable that the real cause of the phenomenon has been determined. A tumor increases its proliferative vigor or manifests an increased adaptiveness as a result of this mutational process. This somatic mutation may occur within a single cell in the neoplastic tissue. Then by the process of selective vitality or adaptiveness of the cells in the mass, the new type of behavior is obtained.

One other point I desire to mention. If one assumes that the spontaneous tumor possesses the same genetic constitution as the host that gave rise to it (and we have every reason to suppose that it does have, since the tumor has arisen by some process or other from cells derived from the body of the normal individual) then we must conclude from our present data, that a tumor mass may deviate from the genetic constitution of the host from which it arose, at least during the process of transplantation.

SUMMARY

1. Mutations or hereditary genetic changes occur within the tumor mass at least during the process of transplantation.

2. These mutations result in a changed physiological activity of the tumor mass.

3. The theory of mutation explains the old phenomenon of the increased percent of successfully growing masses on continued transplantation that has until the present been explained by the theories of adaptation and virulence.

4. The nature of this mutational process is as yet undetermined.

5. The tumor mass may deviate from the genetic constitution of the host that gave rise to it, at least during the process of transplantation.

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HEREDITY OF TWO VARIABLE CHARACTERS IN HABROBRACON

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INTRODUCTION. Review of work on defective venation in Lancaster (L) material.

In the parasitic wasp, *Habrobracon juglandis* (Ashmead), the fourth branch of the radius vein, r_4, has been found to show defects since part or all of it may be lacking in either or both wings. Temperature and other environmental conditions affect the character to a considerable extent but it has been found that when insects are reared at a uniform temperature of 30°C it is possible to estimate hereditary factors with a fair degree of accuracy. Results of studies made on about 95,000 wasps descended from a single female taken in Lancaster, Pennsylvania, (the L material), were discussed at length in a previous publication (WHITING, P. W. 1924). Inheritance is of the sex-linkoid type with exceptions due to patrocliny. (WHITING, P. W. 1921a, WHITING, ANNA R. 1925).

Various ratios of defectives occurred in the different cultures of the L material (WHITING, P. W. 1924, p. 12 ff.) and at least one "mutation" took place affecting this character. Very high percentages did not at first appear but subsequently there were observed fraternities showing upwards of 90 percent defective. From these were isolated "high defective" stocks. (WHITING, P. W. 1924, p. 28 ff.). High defective acted as a unit, *d*, in heredity, the character showing in about 90–95 percent of males and 85–90 percent of females of the stocks and in about 45 percent of the males produced by heterozygous females. Variations in dominance of *D* over *d* occurred in the heterozygous females, *Dd*, so that 5 percent or more of them were defective. "Low defective" and "type" stocks were isolated but "medium defective" was lost. There was evidence that medium reappeared by "mutation" but this "mutant" strain was not retained. (WHITING, P. W. 1924, p. 33). Orange eye color acting as a unit occurred as a definite mutation (WHITING, P. W. 1921a) in the L material. Minor factors for defect causing occasional defectives in low defective stocks and acting as plus modifiers of *d* both for ratio and grade were postulated.

Stock 1, black, and stocks 5 and 8, orange, had normal venation, only 15 defectives occurring in 18,590 individuals, 0.0807 percent defectives.

Stocks 2, 6 and 7, orange, were called low defective stocks, showing about 0.325 percent defectives.

Stocks 3 and 10, orange, and stocks 4 and 9, black, were high defective. Cultures carrying *d* through twenty-eight generations involved fourteen crossings up to type stock alternated with parthenogenetic segregations (WHITING, P. W. 1924, p. 29 ff.). These failed to reduce percentage of defect significantly or permanently. Stocks 3 and 4 showed 94.9 percent defective in males and 90.2 percent defective in females and stocks 9 and 10, isolated at the end of this experiment, showed 94.4 percent defective in males and 89.9 percent defective in females. It may therefore be stated that high defective stocks differ from type stocks by only one significant factor for defective.

The method of origin of defective venation is of interest and is to be considered in the present paper. Its sudden appearance does not necessarily involve mutation in the true sense. Recombination, concentrating subliminal factors, may cause appearance of the character in a higher percentage than previously. It is supposed that there are numerous loci containing factors of different potencies acting in either a plus or a minus direction as regards the production of the character.

For convenience in reference the locus for orange eye may be regarded as in the first linkage group, Chromosome I, while the "main factor" for defective venation, since it segregates quite independently of orange (WHITING, P. W. 1924, p: 31), may be tentatively assumed to be in the second linkage group, Chromosome II. This factor or group of factors acting as a unit may then be called $d_{\mathrm{II}95}$ since it produces about 95 percent defective males in pure stock under standard conditions. Its "normal". alternative, as in stocks 1, 5 and 8 may be called D_{II}. $d_{\mathrm{II}95}$ may have been formed by crossing over of d_{II} groups of lesser potency or by mutation of a single d_{II} unit.

Chromosome I also contains a lethal factor l_{Ia} causing premature initiation of metamorphosis of larvae into pupae, failure to spin cocoons and subsequent death of naked pupae. There were 59 crossovers to 243 straights or 19.5 percent crossovers. Lethal individuals could be classified as to eye color. (WHITING, P. W. 1921b).

Chromosome I also contains factors for defective venation acting as plus modifiers of $d_{\mathrm{II}95}$. One of these of relatively low potency increased both percentage and mean grade of defectives in sons of $D_{\mathrm{II}}d_{\mathrm{II}95}$ females. It may be called $d_{\mathrm{I}a}$. It evidently occurs in stock 1 (WHITING, P. W. 1924, p. 47).

In addition there occurred by "mutation" in Chromosome I a plus modifier of greater potency which may be called $d_{\mathrm{I}b}$. This had the apparently anomalous effect of increasing the percentage of defectives from $D_{\mathrm{II}}d_{\mathrm{II}95}$ females while decreasing their mean grade. The explanation of this apparent contradiction is simply that $D_{\mathrm{II}}d_{\mathrm{I}b}$ males are in part low grade defective while $D_{\mathrm{II}}d_{\mathrm{I}a}$ males are practically all normal.

EXPERIMENTAL DATA

The Iowa City (I) material

Origin and differences from L material

In September 1922 a wild female was taken in Iowa City, Iowa. She produced 145 males and 186 females of normal wing venation. From these were bred the I material which showed several marked differences from the L material.

Fecundity was higher in that there were more offspring on the average per individual female. Either fertilizability of eggs or fertilizing capacity of sperm or both were higher as evidenced by higher female ratio from females mated within the strain and from the fact that reciprocal crosses with L showed higher female ratio than L alone. (WHITING, ANNA R. 1925).

I individuals are more excitable, fly more readily and are more easily lost by inexperienced students.

Crosses demonstrate differences in production of patrocliny. (WHITING, ANNA R. 1925).

Rare occurrence of thelytoky or the production of females from unfertilized eggs, never observed with certainty in the pure L material, has been demonstrated in I material and in crossbred material (LI).

Unpublished data show differences in number of antennal joints.

Different types of occasional freaks occur. There were recorded a very few, 15 males and 2 females, with thoraces showing a ventral split or unusually broadened. Five males had wings markedly reduced in size or venation and eleven had a prothoracic wing or wing sack on one side in addition to the normal primaries and secondaries. The last named type of aberration has never before occurred in studies of Habrobracon, nor have I any knowledge of its appearance in other insects. In eight of the eleven the structure was unexpanded, appearing at first as a sack filled with hemolymph. This later dried and shrivelled. In three it occurred on the right side, in five on the left. In the remaining three of the eleven the prothoracic wing was fully expanded, appearing on the right side in two, on the left side in one. When the wing was expanded the venation was similar to that of the normal primary. The point of origin of this sack or wing was just above but very close to the prothoracic leg.

"Sooty" mesosternum. The mesosterna, especially in the males, averaged much darker than the corresponding area in the L material. The character was called "sooty" and roughly graded as (1) yellow, with very little or no black pigment; (2) gray, with intermediate amount of black pigment; and (3) black, with mesosternum fully or almost fully black. L females are practically all yellow when bred at 30°C or above. I females are lighter than I males but darker than L males as is shown by the following record of material bred under standard conditions.

		YELLOW	GRAY	BLACK	TOTAL
I males	actual	744	486	7175	8405
	percent	8 85	5.78	85.36	
I females	actual	918	402	317	1637
	percent	56 08	24 56	19.36	
L males stocks 4, 5, 10	actual	471	36	20	527
	percent	89 37	6.83	3.79	

Defective venation. As regards defective venation, I material appeared slightly more defective than low defective L.

Approximately 12,678 males were graded, showing 71 or 0.560 percent defective, while 2315 females showed 17 or 0.738 percent defective. L low defective stocks averaged 0.325 percent defective. Breeding tests described below showed I to be very different from L as regards genetic aspects of defective.

Pure I material is being retained, designated as stock 11.

Results of crossing I and L material

Low defective crosses

L orange-eyed normal crossed with I. Color of sternum is not recorded in data presented in this section. Offspring of the original I female were crossed reciprocally with L stock 5 (normal wings, orange eyes).

Stock 5 male with I female gave all type, 63 males and 183 females, while the reciprocal cross produced 71 orange males and 85 type females. F_2 males are recorded in table 1; I×5, with reference to venation and eye color. The percentage of defectives, 1.80, is over three times as high as in pure I and many times as high as L stock 5.

In order to test more fully the production of defectives in the second generation from crosses of I and normal L, wasps from stock 8 and stock 11 were turned over in the spring of 1925 to ACHSA BEAN, a graduate studant at the UNIVERSITY OF MAINE. Stock 8 is an orange-eyed L stock of normal venation showing in the males but 3 defectives among 4336 studied (WHITING, P. W. 1924, p. 38). No change has been noted in this stock. Stock 11 as stated above is of pure I origin.

F_2 males counted by MISS BEAN are recorded in table 1; 8×11. The ratio of defectives, 8.14 percent, is greatly in excess of that for the earlier experiment, 1.80 percent. Both exceed that of the pure I males, 0.560 percent, and of normal L males, 0.0807 percent. These differences may be attributed to chance selection of material used in the crosses.

L orange-eyed low defective crossed with I. An orange male from stock 6 (low defective L, 0.325 percent defective) was crossed with a black I female. Classification was made on the basis of eye color, venation and mesosternum, the latter being called simply yellow or sooty. F_1 showed only normal venation and black eyes, 26 sooty males and 77 yellow females F_2 males are recorded in table 1; 1×6.

Black sooty of mixed origin (LI) crossed with L orange-eyed normal. A sooty male from the F_2 generation just considered was mated to stock 5 female resulting in 25 orange males and 39 black females. All but one of each sex had yellow sterna. F_2 males are recorded in table 1; 5♀×F_2♂ (I×6).

Three defective sooty males from combinations of I material with stocks 5, 6 and 10 were crossed with stock 5 females. There resulted males; sooty 2, orange 104, orange sooty 10, females; type 98, sooty 9. (It may be noted that the two patroclinous males are sooty as well as black-eyed showing paternal character while the majority of the regular males as well as the females have yellow sterna.) F_2 males are recorded in table 1; 5♀ × ♂♂ from I, 5, 6, 10.

In the I×5 series there occurred a male with eyes that were mosaics of black and orange (Freak 154). He bred like a black when crossed to stock 5 females and the F_2 generation from his daughters showed that he transmitted sooty. F_2 males are recorded in table 1; 5 ♀ × mosaic eye ♂ (I×5).

Summary and discussion; failure of linkage of orange, sooty, and defective. The various crosses discussed above involved pure L, stock 5, 6 or 8, on the one side. On the other were black-eyed sooty wasps, either of pure I origin or derived from I crossed with various orange L stocks. Hence the character black-eye and the "main factor" for sooty-sternum came into the cross from one side, orange-eye and yellow-sternum from the other. Among the 4306 F_2 males classified with reference to orange and sooty, there are 49.4 percent recombinations, and hence no evidence of linkage.

As regards defective venation it is impossible to tell the origin of the factors involved. No "main factor" seems to be present since both parents occasionally show defective and the factors may recombine. On the average the I material is somewhat more defective than the L; hence coupling with black or with sooty is to be expected if any linkage exists.

Among the total blacks are 4.032 percent defective and among the total orange 3.105 percent defective. The difference which is slightly less than three times its probable error may possibly indicate the presence of a minor factor for defect, d_1, comparable with such as have been previously shown (WHITING, P. W. 1924, p. 45 ff).

Among the total sooty are 2.506 percent defective and among the total yellow are 3.033 percent defective. This indicates that there is no apparent linkage between the low defective factors and sooty.

Frequencies and ratios are given in table 1.

TABLE 1

F_2 males from black-eyed sooty crossed with orange-eyed yellow, showing segregating relationships of orange, sooty and low defective.

PARENTAL CROSS	STERNUM	BLACK		ORANGE		TOTAL	PERCENT DEF.	PERCENT ORANGE	PERCENT SOOTY
		Normal	Def	Normal	Def.				
I × 5		336	8	374	5	723	1.80	51.04	
8 × 11		512	38	473	49	1072	8.14	48.69	
I × 6	yellow	107	4	91	2	458	4.15	46.29	55.46
	sooty	125	10	116	3				
5♀ × F_2♂ (I × 6)	yellow	36	1	24	1	142	4.23	47.89	56.34
	sooty	35	2	41	2				
5♀ × ♂♂ from I, 5, 6, 10	yellow	334	23	351	13	1439	3.96	50.73	49.90
	sooty	334	18	363	3				
5♀ × mosaic eye ♂ (I × 5)	yellow	445	7	466	7	2267	1.15	49.18	59.20
	sooty	687	13	633	9				
Totals	yellow	922	35	932	23	4306	2.74	49.35	55.60
	sooty	1181	43	1153	17				
	Totals	2951	124	2932	94	6101	3.59	49.60	

Effects of crossing, selection and inbreeding on defective and sooty. The I by 5 series. Origin of stocks 12, 13, 14.

Some of the defective males of the F_2 generation from the original I by 5 cross were mated back to I females and from the progeny lines were continued, the I by 5 series. Selection was made roughly for defective and sooty, both orange and black eye color being retained. Three stocks were isolated all with very dark sterna.

Stock 12 rarely shows defective. Percentages of defectives in the males of the various generations in its ancestry tracing backwards were 2.0, 11.7, 4.6, 1.2, 3.5, 0, 2.4, 0. No definite counts to determine ratio of defectives in pure bred stock 12 have been made, but ANNA R. WHITING in studies of patrocliny recorded venation character in orange males from stock 12 females crossed to black males. They totaled 1968 including 38 or 1.930 percent defective. Stock 12 is therefore a low defective stock.

Relations of stock 12 in production of patrocliny have been discussed by ANNA R. WHITING (1925).

Stock 13 with orange eyes and stock 14 with black eyes were isolated from highly defective lines. Stock 13 had percentages of defective males in various generations as follows: 68.6, 80.0, 51.5, 50.0, 19.5, 33.4, 58.6, 36.9, 15.4, 11.7, 4.6. It joined the common line with stock 12 in the P_4 generation of the latter in which there were 1.2 percent defective males. Stock 14 had 50.0, 41.6, 31.3, and joined the common line with stock 13 in the P_6 generation of the latter in which there were 19.5 percent defective males. U. A. HAUBER (1925) has selected stock 14 for still higher grade of defect and made combinations with d_{1195}, the "main factor" for defective which occurred by mutation in the L material. Thus stocks have been obtained showing practically 100 percent defective.

Stocks 12, 13 and 14 have been continued as "pure" stocks.

Both "sooty" and "defective" showed considerable variation in the I by 5 series. "Sooty" ranged from jet black to clear yellow which is typical of stock 5 as of all L material bred at 30°C or over. The average depth of pigmentation in some lines as in stocks 12, 13 and 14, exceeded considerably pure I material. For the entire series the males showed sterna yellow 162, gray 194, and black 5739, out of a total of 6095. The females showed sterna yellow 1115, gray 1417, black 1123 out of a total of 3655. Percentages of various grades of sooty for I and for the entire I by 5 series are brought together for comparison as follows:

I males.....	yellow	8.85	gray	5.78	black	85.36	Total	8405
I×5 "	"	2.66	"	3.18	"	94.16	"	6095
I females	"	56.08	"	24.56	"	19.36	"	1637
I×5 " . .	"	30.51	"	38.76	"	30.73	"	3655

The I by 5 series is therefore darker with respect to mesosternum color, not only in certain of its branches but on the average, and in both males and females.

Linkage of high defective and sooty

Black-eyed males with sooty sternum and r_4 defective due to low defective factors were crossed to stock 10 females. There resulted 13 black patroclinous males, 170 orange males and 145 black females. F_2 males were graded as yellow or sooty with respect to sternum color, and also classified with respect to orange and defective. Results are recorded in table 2, group A.

A sooty male with normal venation and black eyes occurring in F_2 from stock 6 male crossed with I female was crossed to stock 10 female. There resulted 53 orange-eyed males, 10 normal and 43 defective, graded as yellow 29, gray 20, and black 4. These were typical stock 10 males.

There were also produced 131 black-eyed females of which 3 were defective. All were graded with respect to sterna showing 110 yellow, 17 gray and 4 black. F_2 males are recorded in table 2, group B.

An orange-eyed sooty male from I material bred up to stocks 5 and 6 was crossed to stock 4 female. There were produced males; type 1, defective 66, and females; type 126, defective 2, no sooty appearing in F_1. F_2 males from two virgin F_1 females are recorded in table 2, group C.

An orange defective (grade 8) male derived from combinations of I with stocks 6 and 10 was crossed with stock 11 female. There were produced black males, 1 yellow and 49 sooty, black females, 87 yellow and 8 sooty. F_2 males are recorded in table 2, group D.

TABLE 2

F_2 males from crosses involving orange, sooty and high defective, showing segregating relationships.

GROUP	PARENTAL CROSS	EYE COLOR	TYPE	SOOTY	DEFECTIVE	SOOTY DEFECTIVE	TOTAL		ORANGE	DEFECTIVE	SOOTY	CROSSOVERS (?)
A	10♀ $(oS_{11}d_{11x})$ ×	Black	11	72	62	53						
	♂ $Os_{11}s_xD_{11}d_x$	Orange	9	117	61	44		actual	231	220	286	117
		Total	20	189	123	97	429	percent	53.84	51 05	66.67	27.27
B	10♀ $(oS_{11}d_{11x})$ ×	Black	57	370	286	26						
	♂ $Os_{11}s_xD_{11}$	Orange	58	394	296	30		actual	778	638	820	171
		Total	115	764	582	56	1517	percent	51.29	42 06	54 05	11 27
C	4♀ $(OS_{11}d_{11x})$ ×	Black	17	51	45	2						
	♂ $os_{11}D_{11}$	Orange	18	48	55	1		actual	122	103	102	38
		Total	35	99	100	3	237	percent	51 48	43 46	43 04	16.03
D	11♀ $(Os_{11}s_xD_{11}d_x)$ ×	Black	7	57	48	11						
	♂ $oS_{11}d_{11x}$	Orange	4	57	51	14		actual	126	124	139	36
		Total	11	114	99	25	249	percent	50.60	49 80	55 82	14.46
		Black	92	550	441	92						
	Total	Orange	89	616	463	89		actual	1257	1085	1347	362
		Total	181	1166	904	181	2432	percent	51.69	44 61	55.39	14.88

The four summaries of F_2 males from defective by sooty are totaled with respect to these two characters at the bottom of table 2. It may be seen that there is clear evidence of linkage for the recombinations, crossovers (?), are but 14.88 percent of the total. This may be regarded as a "repulsion" series but since both characters are variable there is no evidence that any of the 362 apparent crossovers are genetic. Moreover any of the 2070 apparent straights may be genetic crossovers. Crossing over could be tested by breeding the apparent crossovers to a parent stock. This test would be complicated by recombination of supernumerary factors both for sooty and for defective.

The "main factor" or group of factors for sooty, showing linkage with d_{II95}, may be called s_{II}. Supernumerary factors for sooty of unknown linkage groups may be called s_X, and similarly supernumerary factors for defective may be called d_X.

Referring to the totals in table 2 we find that out of 2432 F_2 males, orange is very close to the expected 50 percent. There is a marked deficiency of defectives, doubtless due to the character overlapping with normal, despite the presence of supernumerary factors, d_X, in some of the groups. There is a marked excess of sooty, probably due to supernumerary factors, s_X.

Formulae for the parental crosses of the various groups are shown in the second column. Females are from stock 10, ($oS_{II}d_{II95}$) orange yellow defective, stock 4 ($OS_{II}d_{II95}$) black yellow defective, and stock 11 (Os_{II} S_X D_{II} d_X) black sooty sooty-supernumerary normal defective-supernumerary. Males are of diverse composition but always different from females with reference to o, s_{II} and d_{II95}. As regards supernumerary factors, s_X and d_X, their formulae are hypothetical in part although based on origin, character, and progeny.

Orange offspring do not depart significantly from expectation in any of the groups.

Defective is in excess of expectation in groups A and D, s_X coming from the male in A, from the female in D. (It must be noted that expectation is well below 50 percent due to overlapping, and that a ratio of 50 percent or over indicates supernumerary factors, d_X, if temperature is standard, 30°C).

Sooty is in excess in groups A, B, and D, coming from the males in A and B, from the female in D. In groups C and D the males have been freed of supernumerary factors possessed by I material, stock 11, by crossing with L material.

Variation in ratios of crossovers (?) might be correlated with presence of supernumerary factors but this cannot be done satisfactorily in the present instance, because of differences in standard of grading. It would also have been more satisfactory if parental males had been of pure stock.

GENERAL SUMMARY AND CONCLUSIONS

Hereditary factors governing defective venation (breaks in the fourth branch of the radius, r_4) and sooty mesosternum are discussed. Both characters show continuous variation and are much affected by environmental factors, temperature in particular.

In certain stocks of Lancaster material (L) there occurs a factor, d_{II95}, producing about 95 percent defectives among the males. Other L stocks are known as type, (less than one-tenth percent defective), and low defective (less than one percent defective). Total Iowa City material (I) recorded, 12, 678 males and 2315 females, showed less than one percent defective.

F_2 generations from crosses of I material with type or low defective L or with individuals of mixed origin (LI) show no evidence of linkage between sooty and defective.

Certain of these F_2 generations indicate recombinations of factors increasing ratio of defectives. This theory is strengthened by the great increase of defectives in certain branches of a series derived from I crossed with normal L material, I by 5 series. Stocks 13 and 14, derived from the I by 5 series show very high ratios of defectives, over 50 percent, without the presence of d_{II95}.

Sooty mesosternum occurs in I material of which 8405 males and 1637 females were bred and graded in this respect. Males of either L or I material tend to be darker than their corresponding females but I females are darker than L males.

Recombination of factors for sooty is shown by the mesosterna of the I by 5 series and by stocks 12, 13 and 14 derived from it. Stocks 12, 13 and 14 have very black mesosterna in both males and females. Of the I by 5 series 6095 males and 3655 females were graded, averaging darker than the I males and females respectively.

F_2 males from high defective, d_{II95}, crossed with sooty were type 181, defective 904, sooty 1166 and defective sooty 181. Since characters are variable this is not to be considered a correct gametic ratio. It does, however, demonstrate linkage of the main factor for defective, d_{II95}, with the main factor for sooty which may therefore be called S_{II}. This linkage taken in connection with failure of linkage between low defective and sooty possessing s_{II} indicates that the low defective under consideration was not in Chromosome II.

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CHROMOSOME BEHAVIOR IN A CROSS BETWEEN WHEAT AND RYE[1]

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INTRODUCTION

A number of investigators have succeeded in crossing wheat and rye, but only with great difficulty and after many trials. There is a variety of wheat, however, which crosses readily with rye, as high a proportion of pollinations proving successful as in crosses between any two varieties of wheat. This variety I have secured through the kindness of Mr. W. O. Backhouse (1916) of Buenos Aires. By using it as the female parent a large F_1 and a very small F_2 have been produced. From these and from back crosses a great many plants of later generations have been raised. This material, particularly the F_1, has been studied cytologically and the results are reported in the following pages.

Kihara (1919) and Sax (1922) have shown that the cereals furnish an excellent example of polyploidy. The basic chromosome number is 7. Among wheats diploid, tetraploid, and hexaploid species are known, the familiar bread wheats having 21 haploid chromosomes. In rye the haploid number is always 7 so far as known. The hybrids between 14— and 21—chromosome species of wheat show at the heterotypic division 14 bivalent and 7 univalent chromosomes. Those between 7—and 14—chromosome species show 7 bivalents and 7 univalents. It is concluded that in these cases each of the chromosomes of the species with the smaller number finds a mate among the chromosomes of the other species, leaving 7 of the latter unmated. No hybrid between a 7— and a 21—chromosome wheat has been studied cytologically.

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[1] This work was carried on with the aid of a grant from the Research Council of Canada.

Kihara (1924) has given some results of an examination of a single F_1, hybrid between a *vulgare* wheat and winter rye. At the heterotypic division 0, 1, 2 or 3 bivalents were formed, and the number of univalents varied correspondingly between 28 and 22. Some of the univalents divided equationally while others failed to do so. At the homeotypic division a variable number of lagging undivided chromosomes appeared. No material of later generations was available.

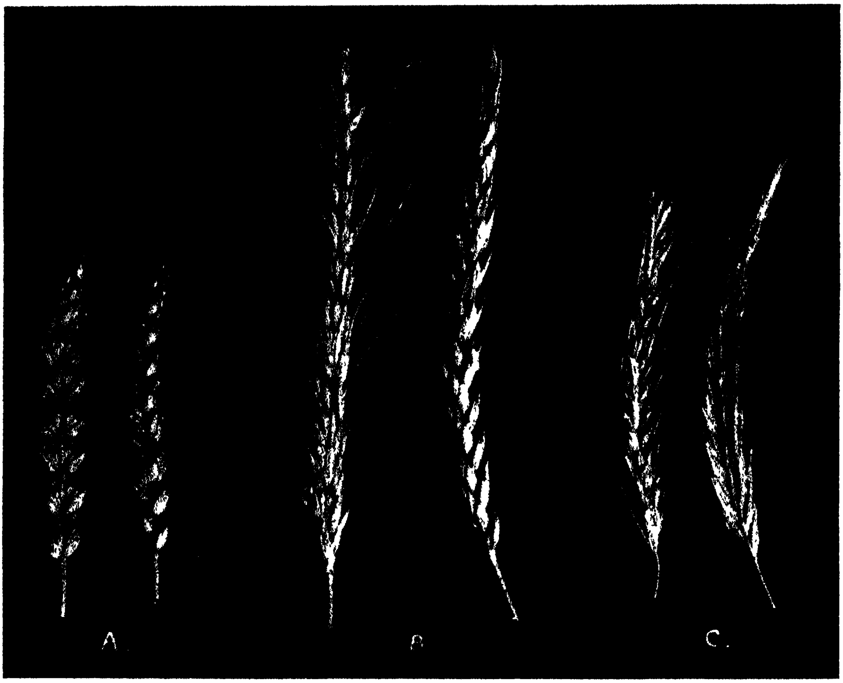

Figure 1.—A, wheat parent; B, F_1; C, rye parent

CHARACTERS OF PARENTS AND HYBRIDS

The wheat used (figure 1, A.) is typical *Triticum vulgare* var, *albidum* Korn. It is a spring wheat of Chinese origin. It shows no peculiarities which would lead one to expect unusual behavior in crossing with either rye or other wheats. The only respects in which it resembles rye a little more closely than do average *vulgare* wheats is in the somewhat more pronounced keel of the outer glume, and the longer, sharper beak of this glume. But many other *vulgare* varieties have these structures as well developed. A marked characteristic of the spike is the sharply incurved, short awns. It behaves like an ordinary variety of *T. vulgare* in crosses with other *vulgare* varieties and with other species of Triticum, except that in certain instances the species crosses are more successful when it serves as one parent.

The rye parent (figure 1,C) chiefly used is a spring type known locally as Prolific. It resembles the variety Petkus. Other sorts were found to cross as readily.

The hybrid (figure 1,B) is very vigorous, at least equalling and often exceeding even the rye parent. Eighteen characters which distinguish the parents were examined. The F_1 is like rye in 2, like wheat in 6, and intermediate in the remaining 10. In regard to 2 of these 10 it is almost exactly intermediate, but resembles rye more than wheat in 3 and wheat more than rye in 5. There is thus a strong preponderance of wheat characters, a fact which may be due to the larger number of wheat chromosomes.

The few F_2 were very variable but for the most part the wheat characters were still more pronounced. A few more rye-like conditions were present and rarely rye characters appeared which were absent in F_1. It is proposed to deal later with the botanical characters in detail and their relation to chromosome conditions.

METHODS

Most of the material was studied by means of smears in iron-aceto-carmine. Many preparations were made of fresh material but the majority were of material fixed in Carnoy's solution and preserved in 70 percent alcohol. The latter method is superior in certain respects; the differentiation is better, and the making of slides and their examination need not be hurried during the short flowering season but may be done at any later time. It is inferior in that the pollen mother-cells do not remain in cylinders but separate, and, when they become flattened after the first division lie in such a position that it is difficult to get polar views. For the material of this study the best reagent was produced by adding 2 drops of a saturated solution of ferric hydrate in 45 percent acetic acid, to 50 cc of a half-saturated solution of carmine in 45 percent acetic acid. The preparations may easily be sealed with asphaltum and then keep excellently for a month or two, gradually becoming darker.

A considerable amount of material was also sectioned in paraffin and stained in Haidenhain's haematoxylin.

CHROMOSOMES OF THE PARENTS

The chromosomes of the wheat are illustrated in figure 2,A and B.[1] They are similar in number (21) and form to those of *vulgare* varieties in general. They are short, thick, and shaped like bent dumb-bells, or rings, or U's or V's with enlarged ends. The equatorial plate is regular and rarely is there even a slight lagging. In anaphase each one splits longitudinally and the ends diverge, so that they appear as 2 sickles or U's attached by the middle.

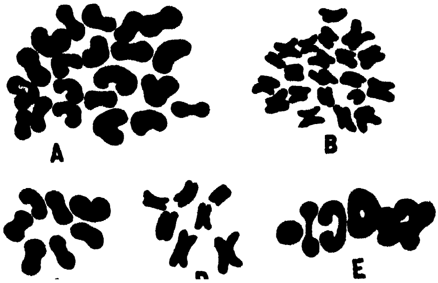

FIGURE 2.—Heterotypic chromosomes of parents. A, wheat metaphase; B, wheat anaphase; C, rye metaphase polar view; D, rye anaphase; E, rye metaphase side view.

The variety of rye used has 7 haploid chromosomes (figure 2, C, D, E,) shaped like those of wheat and behaving similarly. Rye furnishes particularly favorable material since numerous stages are found in one spike, the stamens are very long and easily removed, and the chromosomes few and large.

FIRST REDUCTION DIVISION OF F_1

At the heterotypic division 28 chromosomes make their appearance in the great majority of cases (figure 3A). This is the sum of the haploid numbers of the two parents (rye 7, wheat 21). Evidently no mating of chromosomes occurs in these cases and all that appear are univalent. Occasionally counts of 27 and 26 are made and rarely 25. In these cases shorter, stouter bivalents can usually be distinguished and can be seen dividing in their characteristic fashion (figure 5). Evidently mating of 1 or 2 pairs may take place occasionally and of 3 pairs rarely. In a series of counts made to determine the proportions in which the different numbers occur it was found that 49 cells had no bivalents, 8 had 1 and 2 had 2 bivalents.

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[1] Figure 2 is drawn from paraffin sections. A camera lucida, Zeiss 2 mm apochromatic objective and 25×ocular were used The magnification is reduced 2/3 in reproduction to 1500. Figure 6 was made similarly but with 15x ocular(×950). All other drawings were made from smears in iron-aceto-carmine with 3 mm dry apochromatic objective and 25× ocular (×900).

The individual chromosomes are quite different in form from the normal heterotypic ones of the parents. They are longer, thinner and more curved. Most of them are sickle-shaped, some U- or V-shaped. On the other hand they are not like the vegetative chromosomes nor those of the second reduction division. The latter are still longer and slenderer and almost invariably much curved into V's and I's (figure 8). Why the unmated univalent chromosomes of the heterotypic division should differ from vegetative or homeotypic ones is not clear. The occasional bivalents closely resemble those of the parents, being short, stout rings or dumb-bells.

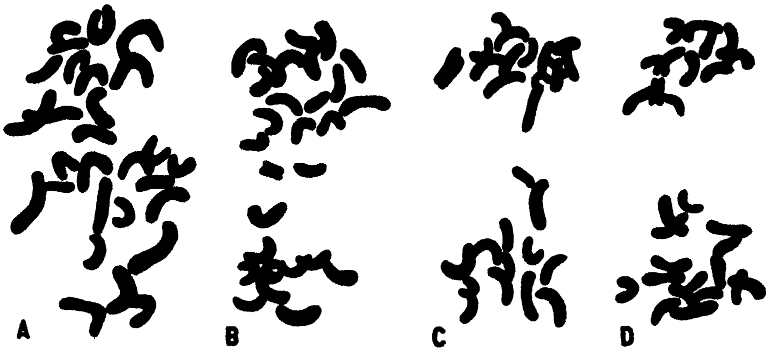


FIGURE 3.—Heterotypic division of F_1, 28 univalent chromosomes separating into 2 groups without division of individuals.

A striking feature of the heterotypic division is the entire absence of an equatorial plate. The shape of the entire group of chromosomes changes from a sphere to an ellipsoid and this becomes longer and narrower by the movement of the chromosomes towards the ends of the cell. The whole group gradually separates into two sub-groups without division of the individual chromosomes (figure 3). There is in fact nothing resembling an equatorial plate stage. The chromosomes simply scatter towards the ends in a long, frequently narrow band. The appearance is therefore very irregular and untidy. Commonly those which reach the end first are reforming a nucleus long before the last ones have reached the end. The separation is usually into approximately equal groups. Usually each group contains 12 to 16, though counts as low as 9 have been made. Apparently the number which goes to either end is determined by the chance disposition of the individuals on either side of the middle line where the equatorial plate would normally form.

Usually before the ends are reached some of the chromosomes show a longitudinal split like that which is seen in the anaphase of the heterotypic division of the parents. This results in the appearance of two parallel rods or 2 slightly curved rods attached at the middle and diverging towards the ends. This appearance is quite different from that of a dividing bivalent. The halves of the latter long remain attached at one end and become much drawn-out. In a considerable proportion of cases nearly all the chromosomes show the univalent split even before the two groups have separated (figure 4). One may find in many cases almost the whole 28 split and still showing no sign of segregation into 2 groups. The two halves of a split chromosome do not normally go to opposite ends but move along together to one end, like the similar chromosomes during anaphase of a normal heterotypic division. Occasionally the halves of one which happens to lie on the equator may move to opposite ends. The usual failure of the halves of a univalent to move to opposite ends is in contrast to the behavior of the univalents in species crosses within the genus *Triticum*. In those crosses the univalents divide at the heterotypic division and wander undivided to one end or the other at the homeotypic. In the Aegilops-wheat hybrids on the other hand SAX (1924) finds that the univalents

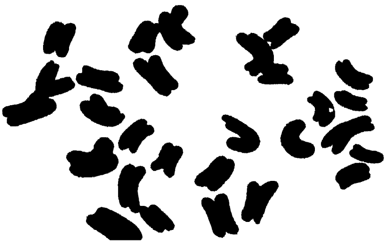


FIGURE 4.—Heterotypic division of F_1 28 splitting univalents.

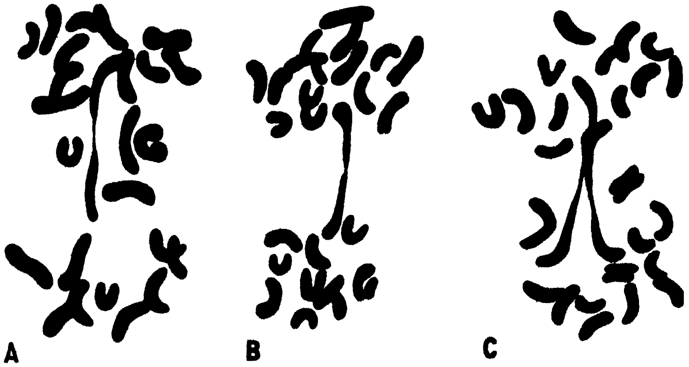


FIGURE 5.—Heterotypic division of F_1 showing dividing bivalents.

usually fail to divide at the first division, though they may occasionally do so.

It has been mentioned that in a percentage of cases only 27 or 26 chromosomes appear and that 1 or 2 bivalents may then be recognizable. These divide in characteristic fashion, the two halves remaining attached at one end for a long time and becoming much elongated (figure 5). The connecting portion becomes drawn out into a thin strand. The bivalents may usually be seen dividing after the univalents have separated at least partially into 2 groups, but they may divide earlier.

Commonly some of the chromosomes fuse and form a daughter nucleus before others have reached the end (figure 6). Late-comers often fuse with each other and form additional nuclei, as many as 4 being seen in some cases. Their size is very variable. Even when several nuclei are present isolated lagging chromosomes are usually to be seen. Commonly 2 or more nuclei formed in this way eventually fuse, and consequently at this stage the nuclei are frequently irregular in outline. Usually one or more of the additional nuclei become cut off by walls, so that previous to the homeotypic division there are frequently 3 or 4 or more cells, some much smaller than others.

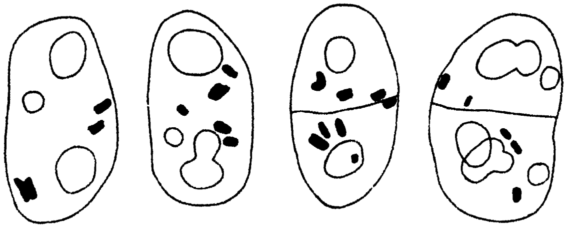


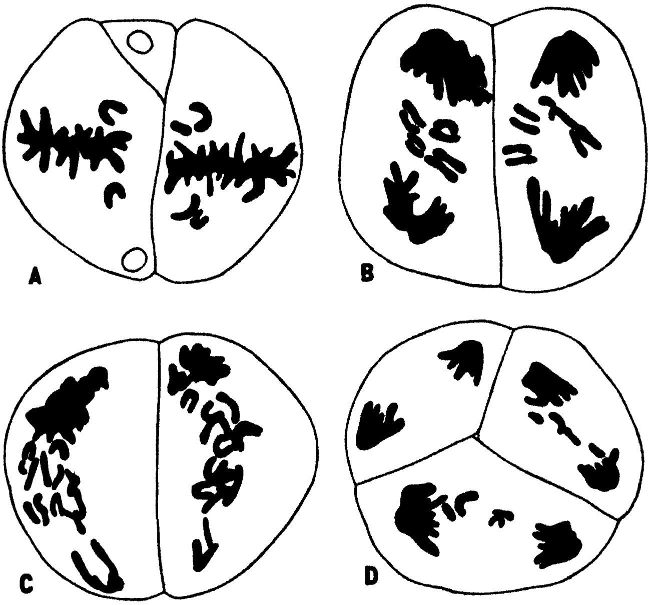
FIGURE 6.—End of heterotypic division.

The prophases of this division may now be considered in the light of the later events. In spite of the facts that no mating of chromosomes usually occurs, and that never more than a few chromosomes are involved in mating, the prophasic events appear to be nearly normal. Though only a preliminary examination has been made of the details of prophases, enough has been done to ascertain that only one contraction occurs. Parallelism of chromatin strands is also evident. If further work shows that this is usual it will have a decisive effect on the parasynaptic interpretation of the similar pairing in the pure species, since in this hybrid little or no mating occurs.

The events at the heterotypic division in Kihara's single hybrid differed in several respects from those just described. Bivalents were present in a larger proportion of the cells. An irregular equatorial plate appeared on which many univalents frequently failed to become arranged. Though actual numbers are not mentioned, apparently a much larger proportion of the univalents divided equationally than in my material.

SECOND REDUCTION DIVISION OF F_1

The homeotypic division is usually much more regular than the heterotypic. A more or less definite equatorial plate is usually formed (figure 7, A). Sometimes all the chromosomes are found in the plate; sometimes 1 or 2 are seen off it; usually a fairly large number fail to reach the plate before the majority have divided. Sometimes no equatorial plate is formed at all (figure 7, C.), and then a very irregular division figure is seen. At this division the chromosomes are long, slender, and much curved or bent. All or nearly all divide (figure 7, B.). Occasionally in individual chromosomes there is no indication of division. These are presumably the occasional univalents which divide at the first division.



FIGURES 7.—Homeotypic division. A, typical metaphase side view; B, lagging chromosomes splitting; C, very irregular division; D, 3 nearly equal cells dividing.

The number of chromosomes varies within wide limits. This variation is a result of the irregular scattering at the heterotypic division, very different numbers often reaching the two ends. It also depends on the fact that frequently more than one nucleus is formed and becomes enclosed in a cell. In those cases in which irregularities are least evident the number varies from 12 to 18. Such cases are represented in figure 8. In the supernumerary cells numbers as low as 2 may be counted.

The extra nuclei usually divide at the same time as the others. One may then frequently find 3 to 5 cells of varying size within the old wall of the mother cell all dividing at the same time, some regularly and some very irregularly. Often small nuclei which are in cells along with large ones fail to divide.

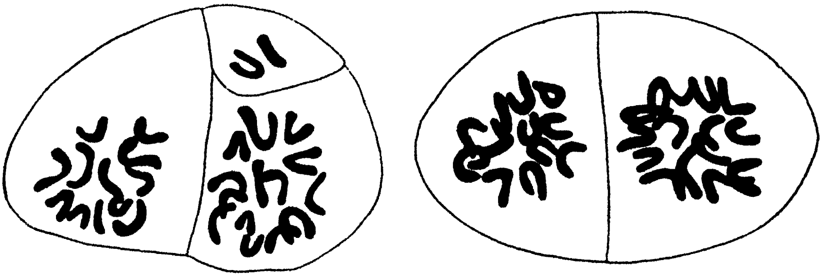


FIGURE 8.—Homeotypic division, polar views.

As in the heterotypic division the chromosomes which first reach the end may form a new nucleus before the later ones arrive. In this way 2 or more nuclei are aften formed and individual chromosomes may remain outside all of them. Often these isolated chromosomes enlarge and form very small nuclei; often they appear to degenerate. There is frequently a fusion of the 2 or more nuclei in a cell.

TETRADS AND POLLEN

Owing to the irregularities at both divisions the resulting "tetrads" usually present a very abnormal appearance (figure 9). While 4 cells may

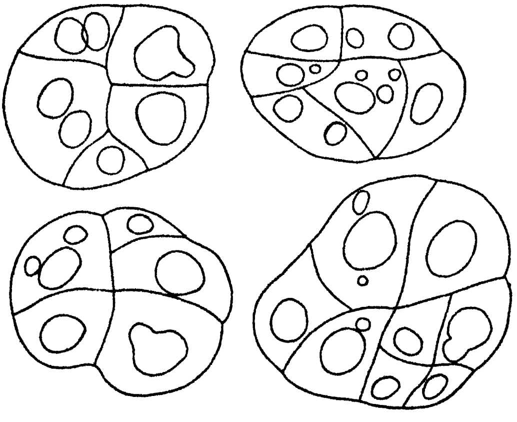


FIGURE 9.—Typical "tetrads" showing extra cells and nuclei and fusion of latter.

commonly be seen in the group, any number from 2 to 10 may occur, "tetrads" of 5, 6 and 7 being very common. The numbers seen in a series of counts were as follows:

Number of cells	2	3	4	5	6	7	8	9
Number of cases	2	4	29	36	26	14	3	2

It is possible that those with 2 were delayed and would still divide but they were seen at a stage when many of the others were already showing degeneration. The two cells were much larger than those of other "tetrads." A number of those with 4 included 2 very small ones. The variation of size of the cells is very great.

Usually some of the cells show more than one nucleus. The number of additional nuclei decreases with maturity, showing that fusion occurs, a point also indicated by the frequently irregular shapes. In many cases, however, more than one nucleus remains in a cell until it shows signs of the death of the protoplasm.

The young pollen grains show indications of abortion while still in the tetrads, and before there is any evidence of their separation. The protoplasm shrinks and takes on a fibrous appearance. The small cells are the first to show these changes.

The mature pollen grains are nearly all empty and shrunken. In a count of 500 in fuchsin and lactic acid only 4 showed well stained protoplasm and this was thin and vacuolate.

CHROMOSOME CONDITIONS IN LATER GENERATIONS

Owing to the scarcity of F_2 plants and the desire to obtain as many seeds as possible no F_2 material was examined. An F_3 plant showed 17 bivalents and 2 univalents, and an F_4 plant of another line showed 17 chromosomes among which no univalents could be distinguished nor any lagging chromosomes. These plants had several distinctively rye characters though for the most part they were like wheat.

From several seeds obtained on unprotected F_1 a large number of descendants have been raised. These were probably the result of natural back crossing with nearby wheat plants, since they were wheat-like in most of their characters, and since the stigmas of F_1 remain fresh and expanded for long periods. Several lines do show some rye characters, however, and it is possible that they were not the result of back crosses but that the more numerous wheat chromosomes dominate the few rye ones. Several plants from these lines were examined. None had fewer than 21 chromosomes at the heterotypic division, some had exactly 21, and some had more The highest number observed was 25 and this included several univalents. In all these cases good equatorial plates were formed. In one case 3 plants from one family all showed 22 chromosomes and no univalents could be detected either by form or lagging. It appears that a line has been started with a higher chromosome number than either original parent had.

A plant produced by artificially back-crossing an F_1 with the wheat parent also had more than 21 chromosomes. It showed at the heterotypic division 21 bivalents and 3 univalents. The behavior was similar to that in species hybrids in wheat.

DISCUSSION

It is evident from the behavior at the first reduction division that each chromosome of *T. vulgare* has a special partner with which it mates in the pure species. Since *T. vulgare* is hexaploid the chromosome set of the ancestor of modern wheats has presumably been triplicated in some way in prehistoric times. Consequently there should be 7 groups of 6 similar chromosomes in the somatic cells and 7 groups of 3 similar ones in the reproductive cells. It might be expected, then, that in pure *T. vulgare* any one of the 6 might mate with any other one. This random mating occurs, according to SAX (1922), in recent examples of chromosome-multiplication in Oenothera, Datura and Primula. In regard to Oenothera this statement is denied by GATES (1924).

In wheat the genetic evidence with multiple factors indicates that the assortment is not random. The behavior at the heterotypic division in these wheat-rye hybrids confirms that conclusion. None of the wheat chromosomes usually finds a mate though there should be 3 of each kind, even if the rye chromosomes are disregarded. The usual absence of mating shows that in pure wheat each chromosome has its own particular partner. As SAX (1922) has suggested the chromosomes may have differentiated so much since the original triplication that random mating is no longer possible. Since all wheat species were distinct in prehistoric times (PERCIVAL 1921) there has been sufficient time for the differentiation.

The occasional presence of 1 or 2 bivalents may, however, indicate that some of them are able to mate occasionally with chromosomes which are not their normal partners, if those partners are absent. Of course a bivalent may also be the result of mating between a rye and a wheat chromosome. But it would seem more probable that chromosomes which have been duplicated within the genus *Triticum* would mate with each other than with chromosomes from another genus. Furthermore, WINGE (1924) has given evidence that in pure *vulgare* wheat a chromosome may occasionally mate with one which is not its normal partner, and that one of the results is a speltoid mutation. If such a mating may occur when the partner is present it is still more likely to occur when the partner is absent. But the usual absence of bivalents in the wheat-rye hybrids, and their very small number when present, show that wheat chromosomes usually remain faithful even to their absent mates.

COLLINS and MANN (1923) have shown that in species hybrids in Crepis the behavior is similar. In the F_1 of *Crepis capillaris* (haploid 3) and *C. setosa* (haploid 4) no mating occurs and the 7 univalent chromosomes pass undivided to the poles. In the hybrid between *C. setosa* and *C. biennis* (haploid 20) the *biennis* chromosomes all mate in 10 pairs leaving the *setosa* ones unmated. Though the wheat-rye cross is much wider botanically the chromosome behavior is intermediate between those two Crepis types since an occasional mating occurs.

The only other genus cross which has been carefully studied cytologically is the one between *T. vulgare* and *Aegilops cylindrica*. SAX (1924) finds the behavior in this much more regular. *Aegilops* has 14 haploid chromosomes. In the F_1 about 7 bivalents and 21 univalents are usually formed. Supernumerary nuclei and cells are very rare. SAX suggests that 7 Aegilops chromosomes mate with 7 wheat ones and that 14 wheat and 7 Aegilops ones remain unpaired. In some cases he was not able to find the full 7 bivalents nor in many cases the full 21 univalents.

In view of their behavior in Aegilops-wheat hybrids, in rye-wheat hybrids, and in species hybrids within the genus Triticum, it would appear that the chromosome relationships in wheat and its relatives would be as follows:

	Sets of seven chromosomes				
	A	*B*	*C*	*D*	*E*
T. vulgare	*x*	*x*	*x*		
T. durum, dicoccum, etc.	*x*	*x*			
T. monococum	*x*				
Aegilops			*x*	*x*	
Secale					*x*

The three sets of 7 haploid chromosomes of *T. vulgare* are designated A, B and C. Two of these mate with the 2 sets of the emmer types of wheat which may then be designated A and B. One of these latter mates with the single set of einkorn which may therefore be designated A. Set C will then be distinctively *vulgare*. The set in Aegilops which mates with one set in *T. vulgare* will probably be C since, as Percival has pointed out, the important characters which distinguish the *vulgare* from the emmer type are all found in Aegilops. If this is true the other set in Aegilops must be incompatible with either A or B; otherwise it would mate with one of them and 14 bivalents and 7 univalents would be found. This different set may then be designated D. (The existence of a distinct set would have to be assumed even if the Aegilops set which mates with *vulgare* chromosomes be considered A or B). Therefore two 14-chromosome types, Aegilops and emmer wheat, have all their chromosomes incompatible. Nevertheless TSCHERMAK (1913) was able to cross Aegilops with all species of wheat. Since there is no botanical or genetic reason for thinking that the rye set is similar to Aegilops set, and since it does not mate with A or B or C it must be considered different from all the others. There is not sufficient evidence to decide where all these differentiations occurred or how the multiples were produced in different lines. But further combined genetic and cytological work on species and genus crosses should throw light on the problem.

It is possible that a definite set of 7 Aegilops chromosomes does not always mate with a set of 7 *vulgare* ones. Occasionally SAX could find no more than 5 bivalents and frequently less than 21 univalents, so that there may be a variable amount of mating. This may correspond to the variable though smaller amount of mating in wheat-rye hybrids. The latter is probably between wheat chromosomes only.

This work shows, however, that great caution must be exercised in drawing conclusions regarding relationships from ease of crossing or chromosome numbers. This particular variety of wheat crosses with rye as easily as with any other species of wheat, much more easily than with some species, and quite as easily as any variety of *vulgare* crosses with any other variety. It is not difficult to get 90 percent of pollinated flowers setting seed, while JESENKO (1913), working with a number of *vulgare* wheats, secured only 35 seeds from 6129 flowers pollinated with rye (and 12 of those from 1 head). Einkorn wheat which has the same number of chromosomes as rye (7) cannot be crossed with rye, while this particular *vulgare* variety with 3 times the number may be crossed readily. Similarly it has been found almost impossible to cross a certain variety of *T. dicoccum* (14 chromosomes) known as Khapli, with *T. vulgare* though numerous attempts have been made, owing to its high rust-resistance. Very few seeds are obtained, and these few either fail to germinate or produce F_1 which fail to reach maturity. Other varieties of *T. dicoccum* cross readily with *T. vulgare*, and the F_1 shows a high degree of fertility. It is evident that a large number of varieties must be used in species crosses before final conclusions can be drawn.

It is also evident that conclusions regarding economic results must not be too hastily drawn from a consideration of chromosome numbers. It appears that some of the chief advances in wheat breeding depend on the crossing of different species—a procedure which presents great difficulties because of the differences in chromosome numbers. But it is clear that very different results may be expected when different varieties of the same species are crossed with a different species. In the present example we have a large number of lines in late generations with a preponderance of wheat characters but also certain definite rye characters. Judging from chromosome numbers and experience with other attempts to cross wheat and rye, this would be impossible.

The rare production of seed on selfed F_1 plants and the chromosome numbers of the F_3 and F_4 examined show that viable plants are occasionally produced without the full complement of wheat chromosomes. The botanical characters of these plants also show that they carry rye chromosomes. The absence of some *vulgare* chromosomes has not tended in these cases to produce *emmer* characters.

The considerable proportion plants of later generation resulting from back-crosses, which had more than 21 chromosomes is of interest. In all these cases there were at least 21 bivalents; in most of them there were 21 bivalents and a number of univalents; in one case a stable condition with 22 bivalents appears to have been reached. It would be of great interest both theoretical and economic to produce a stable race of wheat with more chromosomes than any now in existence. It is not easy to see how plants with more than 21 chromosomes have arisen immediately following a back cross, unless the events in the female may occasionally be different from those in the male. If the chromosomes in the megaspore mother cell did not scatter undivided as in the microspore mother cells, or if rarely the division is so irregular that only 4 or 5 chromosomes go to one end, and all the others to the opposite end, the result might be achieved, though even in the last instance 21 must be wheat chromosomes since 21 bivalents are formed in the offspring. If, as in the lily and numerous other angiosperms (COULTER and CHAMBERLAIN 1903) the megaspore mother cell could develop directly into an embryo-sac without forming megaspores, then the fusion of 2 nuclei from opposite ends of the female gametophyte for endosperm formation would restore approximately the full haploid complement of rye and wheat chromosomes. Such a fusion nucleus might be expected after fertilization to grow into an embryo instead of endosperm, since its chromosome complement is like that of an egg. According to WATKINS (1925), however, 4 megaspores are produced in wheat and only one functions. What happens in rye is unknown.

It is not so difficult to account for the appearance of plants with more than 21 chromosomes in later generations. If an F_1 female gamete with 18 chromosomes (a number seen at the homeotypic division in pollen mother cells) including 3 rye ones, is fertilized by a male gamete with 21, the heterotypic division in the offspring would be expected to show 15 bivalent wheat chromosomes, 6 univalent wheat and 3 rye. Occasional gametes may then be expected to have 24 chromosomes (21 wheat and 3 rye) which being fertilized by 21 chromosome gametes from the same plant or pure wheat would give the desired condition.

SUMMARY

A certain variety of *vulgare* wheat crosses as easily with rye as with any other *vulgare* variety and more easily than with other species of wheat. The proportion of successful pollinations is as high as in crossing any two varieties of wheat. The F_1 is very vigorous and not quite completely sterile.

The wheat used has the typical 21 haploid chromosomes of *Triticum vulgare* and the rye has the typical 7 of Secale.

At the first reduction division in F_1, 28 univalent chromosomes usually appear. These move without dividing to opposite poles, nothing like an equatorial plate being formed and the division figure appearing highly irregular. Often 1 or 2 and rarely 3 bivalents appear and divide in characteristic fashion. Supernumerary nuclei are formed from lagging chromosomes; these may fuse with the principal nucleus, or may become enclosed in cells of their own, or may remain unfused in the same cell as the principal nucleus.

The prophases of this division appear from preliminary observations to show only one contraction. Some parallelism of chromatin strands is evident though not expected on the parasynaptic interpretation.

The second division is usually much more regular than the first, but may be quite as irregular. An equatorial plate is usually formed on which nearly all the chromosomes become oriented and divide, though lagging chromosomes are common. "Tetrads" usually have from 4 to 8 cells but may have more or fewer.

From back-crosses plants with more than 21 chromosomes have been secured.

In tetraploid and hexaploid species of wheat each chromosome has a single mate; random mating amongst duplicated chromosomes does not occur normally, but may occur abnormally. It is probable that there is among wheat chromosomes no mate for any rye chromosome.

Caution is necessary in drawing conclusions concerning relationships from ease of crossing or chromosome conditions.

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MUTATIONAL MOSAIC COAT PATTERNS OF THE GUINEA PIG

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INTRODUCTION

Most of the guinea pigs which one sees have a mosaic coat pattern of some sort. The piebalds have a pattern of colored spots on a white ground, the tortoiseshells have a pattern of dark spots on a yellow ground and the tricolors have both yellow and dark colored spots of some sort on a white ground. It has been suggested (EYSTER 1924) that these patterns may be due to somatic mutations of an unstable gene, such as has been demonstrated in variegated corn. There certainly appears to be something akin to mutation as far as cell lineages are concerned. On the other hand, the more or less orderly relationship to the parts of the body indicates that the pattern is determined primarily by developmental processes of the type responsible for ordinary regional differentiation. The genes for piebald (s) and tortoiseshell (e^p) are simple recessives, seemingly as stable as any other genes in the germ-plasm. THE BUREAU OF ANIMAL INDUSTRY has maintained a number of inbred strains of guinea pigs since 1906. One of these families (No. 34) was tortoiseshell (SSe^pe^p) throughout its history. All of the others have bred consistently tricolor (sse^pe^p) except that in one of them (No. 13) true breeding red-white piebalds ($ssee$) segregated out in one line near the beginning. Some 35,000 guinea pigs have been recorded in these lines and from crosses between them, but there is not the slightest evidence that the piebald factor has ever mutated to its dominant allelomorph (S) or that the tortoiseshell factor has mutated either to dominant true-breeding black (E) or to the recessive true breeding red (e). Black-white and red-white bicolors appear not infrequently but breeding tests have indicated that these differ genetically in no respect from their tricolor relatives, their behavior contrasting sharply with that of blacks (EE) and reds (ee) obtained from other sources. With this type of mosaic pattern we shall have little further concern in this paper.

There are five sets of allelomorphs in guinea pigs which normally produce a uniform change in color throughout the coat except that the degree of effect may show an entirely regular regional differentiation. Mosaics in the effects of these factors are extremely uncommon, but when they do occur they presumably indicate some sort of a mutational or chromosomal change in the region affected. The purpose of the present paper is to give an account of the seven possible cases of this sort which the writers have noticed among over 40,000 guinea pigs which they have recorded.

The record of every guinea pig born in the experiments includes a sketch of the pattern made in a rubber stamp outline, similar to the patterns shown in the figures. These drawings have been made for the most part by the junior author, who has been on the lookout for any irregularities of the kinds to be described. The senior author has gone over all of the animals born each week for the purpose of recording the intensities of the various colors. Other opportunities for observation occur at the time of weaning and at times when weights are taken. Doubtless a few inconspicuous cases have been overlooked, especially in animals born dead. We feel safe in saying, however, that such mosaics are extremely uncommon in guinea pigs.

THE COLOR FACTORS OF GUINEA PIGS

The color factors which are known to have been present in this stock are given below. The black-eyed golden agouti variety with all 7 dominant factors is taken as the type, as having the factorial composition of the wild Cavia cutleri in these respects. In this variety each hair is black with a "red" (*ochraceous tawny*) subterminal band. This band is wider and lighter in color on the belly, which thus has a yellow appearance.

S, s $\quad$ s = piebald (white areas). Incomplete dominance.
$E, e^p, e.$ $\quad$ e^p = tortoiseshell (red areas).
e = self red

A, a^r, a a^r = narrow red ticking, ticked belly
a = self black
C, c^k, c^d, c^r, c^a, c^k = slight dilution of black, marked dilution of red
c^d = marked dilution of both black and red
c^r = slight dilution of black, red replaced by white, eyes red
c^a = black reduced to sooty white, red replaced by white, eyes pink (albino)

In all cases, intensification of black, but slight fading of yellow on exposure to cold and with age. Incomplete dominance in compounds of lower members of this series.

F, f. f = dilution of red only (except in presence of p), effect increasing markedly with age.
B, b. b = brown in place of black; dark red eyes. No effect on red.
P, p. p = pale sepia in place of black; pink eyes. No effect on red.

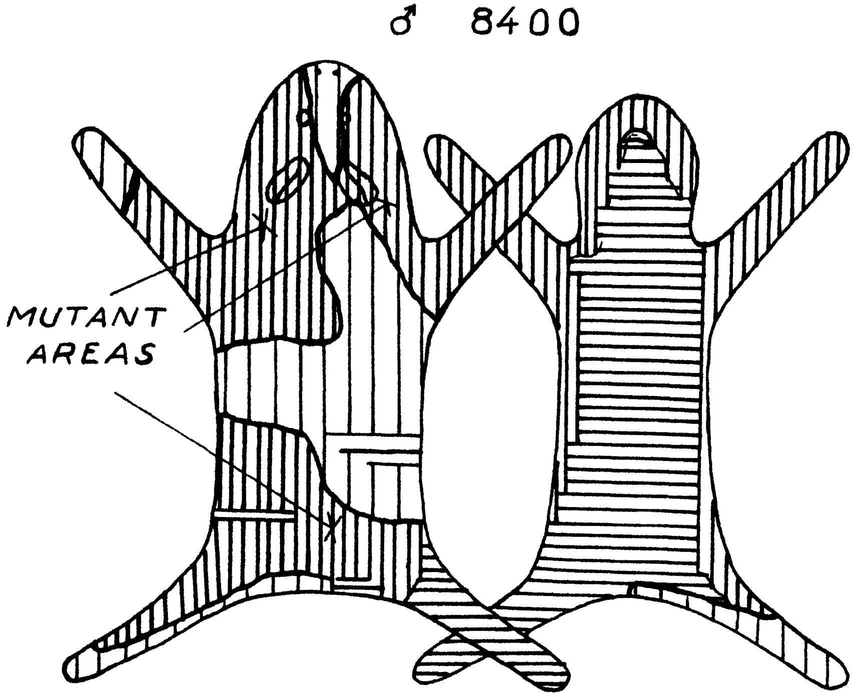

FIGURE 1.

The coat pattern of ♂8400. Vertical lines represent the agouti and horizontal lines the red or yellow of his tortoiseshell pattern. The heavily shaded portion represents the mutational intense portion (golden agouti and red) while the light shading represents the normal yellow agouti and yellow.

As already noted mutational mosaics could not easily be detected in piebalds (ss) or tortoiseshells (e^pe^p) because of their naturally mosaic patterns. The mosaics to be described apparently involved A in one case, the C series in one case certainly and possibly in two others, F in two cases and P in one case. In only one case is there evidence that the germ-plasm was affected. We will describe this case first.

A SOMATIC AND GERMINAL MOSAIC ($Cc^d+c^dc^d$)

Male 8400 attracted attention at birth (December 23, 1918) through having a mosaic pattern of red agouti and yellow agouti. There were two separate red agouti regions as shown by the heavy bars in figure 1. One of these included the head about the eyes and ears, the shoulders and right fore leg. The other included both flanks and the right hind leg. Thus, the nose, left fore foot, a belt around the middle (extending forward on the right shoulder) and finally the left hind leg and left side of the rump were yellow agouti. The pattern was sufficiently striking at birth but became less easy to recognize later, largely, apparently, because of a relatively greater amount of fading of the red agouti parts. On brushing back the hair it may be seen that the black as well as the red is more intense in the red agouti areas. The differences are such as would be expected between an intense agouti (C-) and a dilute agouti (c^dc^d)[1]. Approximately 60 percent of the coat is red agouti dorsally. The entire belly appears yellow, but as there is little difference in intensity between "intense" and homozygous dilutes in this region, it is probable that the mutant patches extend over part of it.

This animal attracted special attention from the first because of the fact that both parents were dilutes and thus should not transmit the dominant intense coloration which he clearly showed. The sire (♂4849) was a self yellow of grade 8, believed to be c^dc^d with c^kc^d as a possibility. He was mated twice. His first mating was with a black-cream tortoiseshell necessarily c^kc^r from her parentage. The progeny consisted of 3 with yellow in the fur (c^kc^d) and one with cream (c^dc^r). His second mating was with a dilute brown (♀6466) which later was proved by breeding tests to be c^dc^r. This mating produced 9 with yellow in the fur (c^dc^d) and 4 with cream (c^dc^r) in addition to the mosaic male (♂8400) showing both red and yellow which was thus assumed to be genetically c^dc^d. The color of the sire, together with his breeding record of 17 dilute young, from dilute females, omitting the intense dilute mosaic, agree in indicting that he was a homozygous dilute. His own sire, however, was an intense (agouti-red-white) necessarily Cc^d as coming from a cross between a yellow male (c^dc^d) presented by Professor W. E. CASTLE and a golden agouti tricolor female of an inbred family (No. 39) which had always been CC. His dam was a cream that might have been either c^dc^r or c^kc^a as coming from a mating between a cream, c^dc^a, presented by Professor CASTLE and a black-cream c^kc^r. from a mating between a red-eyed brown (c^rc^r) presented by Professor CASTLE and a black-yellow from an inbred family (No. 18) homozygous c^kc^k in the branch concerned. The brothers and sisters of ♂4849 were 3 with red (C-), 1 with yellow ($c^{kd}c^d$) and 3 with cream (c^dc^{ra}) in harmony with the formula assigned his parents.

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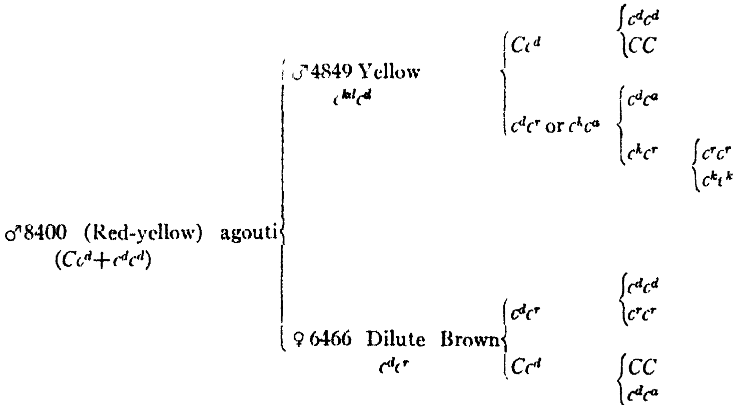
[1] The colors of the different albino series compounds are described in detail elsewhere (Wright, 1925). The intensity of yellow is the best criterion. Using compound symbols, C– is typically red of grade 10 or 11, $c^{kd}c^{kd}$ yellow of grade 6 to 8, $c^{kd}c^{ra}$ cream of grade 4 or 5 and $c^{ra}c^{ra}$ pure white.

The possibility that ♂4849 may have transmitted C from his father must of course be borne in mind, but the assignment of formula Cc^d to him raises difficulties in accounting for his own color and his failure to sire any unequivocal intense among 18 young, without assisting much in accounting for his mosaic son.

Going back to the dam of the mosaic, ♀6466 was a brown, a color in which the effects of the C series are not easily distinguished. She was mated three times, however, with results that make it certain that she was c^dc^r. Her first mating was with ♂4849, in which, as above noted, she produced 9 young with yellow (c^dc^d), 4 with cream (c^dc^r) in addition to the mosaic. Her second mating was with an albino male (c^ac^a) made especially as a test. She produced two sepia-cream young clearly c^dc^a from their color, 2 sepias proved to be c^dc^a by breeding tests, and 2 red-eyed sepias, clearly c^rc^a from coat and eye color. Her third mating was with a red-eyed dark sepia (c^rc^r). She produced 9 black-eyed dilutes (c^dc^r) and 9 of the appearance of homozygous red-eyed dilutes (c^rc^r). She thus had 38 graded young to 23 of which including the mosaic she supplied c^d and to 15 of which she supplied c^r. It is perhaps worthy of record that she produced her 16th litter at the advanced age of five years and one month. She died a month later. Like ♂4849, she had an intense parent but in view of her extensive breeding record in which it was proved conclusively that she transmitted both c^d and c^r, the possibility that she transmitted C seems negligible. The formulae of the ancestors of the mosaic are shown below:



Before leaving the ancestry, it may be well to state that a careful investigation was made at once to determine the possibility that the parentage of the mosaic had been incorrectly assigned. He was born in his parents' second litter, with two litter mates, a sepia-yellow (c^dc^d) and a sepia-cream (c^dc^r). There was no evidence that any intense guinea pigs had been in the pen in which he was found except his reputed parents for seven months. Female 6466 undoubtedly had a litter at this time. Breeding tests showed that the mosaic transmitted e and b, factors necessarily transmitted by ♂4849 (ee) and ♀6466 (bb) but which could have come from only a small number of other animals in the stock, descended from a few animals presented by Professor CASTLE. It happened that all but one of these other matings produced a litter within two months before or two months after. The one exception had been sterile for a year and could have produced the mosaic only through a double mutation. There is thus exceptionally complete evidence in this case that there was no mistake in parentage.

The genetic constitution of the mosaic was tested by matings with albinos (c^ac^a) red-eyed dilutes (c^rc^a) and one dark-eyed sepia (c^dc^a). The matings with albinos and red eyes were desirable since the distinction between the red of Cc^{ra} and the cream of c^dc^{ra} is more striking than that between the red of Cc^d and the yellow of c^dc^d. In order to obtain as many young as possible he was moved in a ten-week cycle through three pens, in each of which there were usually two or three females.

The litters are shown in table 1 in chronological order. The first litter contained a golden agouti (intense) in addition to a cream agouti, thus proving that he transmitted intensity. The next litter confirmed this conclusion. Altogether, he produced 79 intense young and 149 dilutes. The expectation on the basis of his parentage and the appearance of the lighter areas in his coat was 100 percent dilutes. If, however, he were a heterozygous intense (Cc^d) the expectation would be 114 intense to 114 dilutes. The actual result is, of course, wholly out of harmony with the first hypothesis and deviates from the second by 35 ± 5.0, or seven times the probable error. The chance of obtaining such a deviation in this direction is about 1 in a million. Extensive experiments with the albino series have given no other significant departures from the expected ratios. Thus it seems safe to conclude that ♂ 8400 did not breed like a normal Cc^d. The obvious suggestion is that he was a mosaic germinally as well as somatically. If his germinal epithelium were 70 percent Cc^d and 30 percent c^dc^d, the observed ratio would be obtained. The dilute progeny and

TABLE 1

The breeding record of ♂8400. All of the females with which he was mated were as indicated albinos (c^ac^a) or red eyed dilutes (c^rc^a) with one exception, No. 8084, a dark eyed dilute (c^dc^a).

DATE OF BIRTH	DAM		INTENSE	DILUTE
	No	formula		
1919 Sept. 2	215	c^ac^a	1	1
Nov. 5	215	c^ac^a	1	1
Nov. 30	8084	c^dc^a	2	2
Dec. 17	752	c^rc^a	1	1
Dec. 29	9154	c^rc^a		1
1920 Jan. 23	1317	c^rc^a		2
Jan. 28	8203	c^ac^a	1	3
Feb 23	752	c^rc^a		1
Mar. 9	215	c^ac^a	1	1
Mar. 31	1317	c^rc^a	1	1
Apr. 9	8084	c^dc^a	1	3
Apr. 29	752	c^rc^a		3
May 29	215	c^ac^a	1	2
June 9	8203	c^ac^a	1	3
July 3	9154	c^rc^a		1
July 6	752	c^rc^a		3
Aug 4	1317	c^rc^a	1	2
Aug 7	215	c^ac^a		1
Sept 4	4496	c^rc^a		1
Sept. 9	4497	c^rc^a		1
Sept 10	752	c^rc^a		2
Sept 10	9154	c^rc^a		2
Oct. 18	2	c^rc^a	2	2
Oct 30	8203	c^ac^a	1	3
Nov 14	4497	c^rc^a	3	1
Nov. 18	752	c^rc^a		1
Dec. 6	215	c^ac^a	1	
Dec. 15	1317	c^rc^a	1	1
Dec. 24	2	c^rc^a	1	4
Dec 26	4496	c^rc^a	2	1
1921 Jan. 23	752	c^rc^a	1	1
Mar. 27	7523	c^ac^a	1	1
Mar 28	4497	c^rc^a	1	2
Apr. 1	752	c^rc^a	1	2
Apr. 20	1317	c^rc^a	2	1
Apr. 23	215	c^ac^a	1	1
May 7	7970	c^ac^a		2
May 8	7928	c^ac^a		3
May 13	8203	c^ac^a		1
June 7	752	c^rc^a		2
July 5	1317	c^rc^a		1
July 13	7928	c^ac^a	5	1
Aug. 5	7533	c^ac^a	1	3
Aug. 13	752	c^rc^a	2	2
Sept. 17	8203	c^ac^a	3	1
1921 Sept. 20	7928	c^ac^a	1	2
Oct. 14	7533	c^ac^a	3	1
Oct. 19	752	c^rc^a	1	2
Nov. 1	1317	c^rc^a	1	2
Nov. 25	8203	c^ac^a	1	4
1922 Feb. 8	7928	c^ac^a	1	2
Feb. 26	752	c^rc^a		2
Mar 5	7533	c^ac^a		3
Mar 31	1305	c^ac^a	2	2
May 20	752	c^rc^a		2
Sept. 5	7928	c^ac^a	1	1
Sept 26	8203	c^ac^a		1
Sept 27	752	c^rc^a	2	2
Oct 10	14963	c^ac^a		1
Nov 15	7045	c^ac^a	1	1
Nov. 22	14789	c^ac^a		2
Dec 14	7928	c^ac^a		3
1923 Jan 7	752	c^rc^a	1	2
Jan 8	14963	c^ac^a		1
Feb 14	14962	c^ac^a	1	1
Feb 20	7928	c^ac^a	1	1
Mar 14	752	c^rc^a	1	3
Mar 14	14963	c^ac^a		
Mar 20	16355	c^ac^a		2
Apr 7	7045	c^ac^a	2	1
May 19	14963	c^ac^a	1	2
May 26	16355	c^ac^a	1	1
July 4	14962	c^ac^a		2
July 9	7928	c^ac^a		2
Aug 1	16355	c^ac^a	2	1
Aug 13	17608	c^ac^a	1	2
Aug 25	7045	c^ac^a	1	4
Sept 11	7928	c^ac^a	3	2
Sept 20	14963	c^ac^a		3
Oct 1	752	c^rc^a	1*	
Oct 5	16355	c^ac^a	1	2
Nov 26	14962	c^ac^a		3
Dec 9	752	c^rc^a	5	1
Dec 9	14963	c^ac^a		
Dec 12	16355	c^ac^a	1	3
1924 Jan 18	17608	c^ac^a	1	2
Total			79	149

*A "black-white" born dead is not included, as it might have been either Cc^{ra} or c^dc^r.

their descendants showed grades of sepia which indicated that the dilution factor which he transmitted was always c^d rather than c^k.

There is some indication that he did not produce the same ratio of intense to dilute at all times. Up to the end of 1919, his record was 5 intense to 6 dilute, giving no indication that he was breeding differently from Cc^d. During the next nine months, however, he produced only 7 intense to 32 dilute. The total results, summarized to this point, deviated from equality by 5.4 times the probable error and were presented by the senior author at the meeting of the American Society of Naturalists in 1920 as fairly conclusive evidence of an aberrant ratio. There followed, however, a period of thirteen months in which his record 34 intense to 42 dilute showed a much less impressive departure from equality. Indeed, in one four-month period, July to October, 1921, an excess of intense (16:13) was produced. During the next seventeen months there was again a marked deficiency of intense: 13 intense to38 dilute. The final ten months with 20 intense to 31 dilute, seemed to show another shift toward equality. These variations in ratio can only be taken as suggestive, however, since on comparing with the expected figures on the basis of a constant proportion (34.7 percent) of intense, we obtain a value of P of .125 for the χ^2 test. Thus irregularities as great as those observed would be expected to occur one time in eight purely by chance. Another test can be obtained by comparing the record of 34 intense to 42 dilute produced in 13 months with the entire remaining record. The result is a contrast of 44.7 ± 3.8, with 29.6 ± 2.5 percent, a difference of 15.1 ± 4.6, about 3.3 times the probable error which might be expected to occur about 1 time in 35 by chance. Neither of these tests is quite fair in that they involve a selection of the periods for comparison. Nevertheless, there is a distinct suggestion that the proportion of germ cells carrying intense and dilute varied from time to time. This could be explained on the hypothesis that the germinal epithelium was mosaic and that the relative activity of different portions varied. A belated attempt of an experimental test of this point was made by removing the left testis (October 18, 1923). Only one litter was sired following the operation. This litter included 1 intense to 2 dilute, proving that both C and c^d were transmitted by the germinal epithelium of the right testis. Male 8400 died, (December 30 1925) at the rather advanced age of seven years, but produced no young since the litter referred to above, although mated with eight females for much of the time.

A number of his progeny have been tested by mating with albinos or red eyes, or with each other. There is nothing unusual in their records. Six intense young mated with albinos or red eyes have produced 46 intense to 59 albinos or red eyes, a ratio which does not depart significantly from equality. Two matings between intense young have produced a total of 24 intense to 8 albinos, exact expectation. One homozygous dilute son, from the one mating in which ♂8400 was paired with a dilute, has produced 9 typical dilute young in a mating with an albino. Two heterozygous dilute sons mated with albinos have produced 8 dilute to 5 albinos where equality is the expectation. A mating between two dilutes produced two dilutes. The intense grandchildren of ♂8400 appear to be exactly like ordinary intense animals.

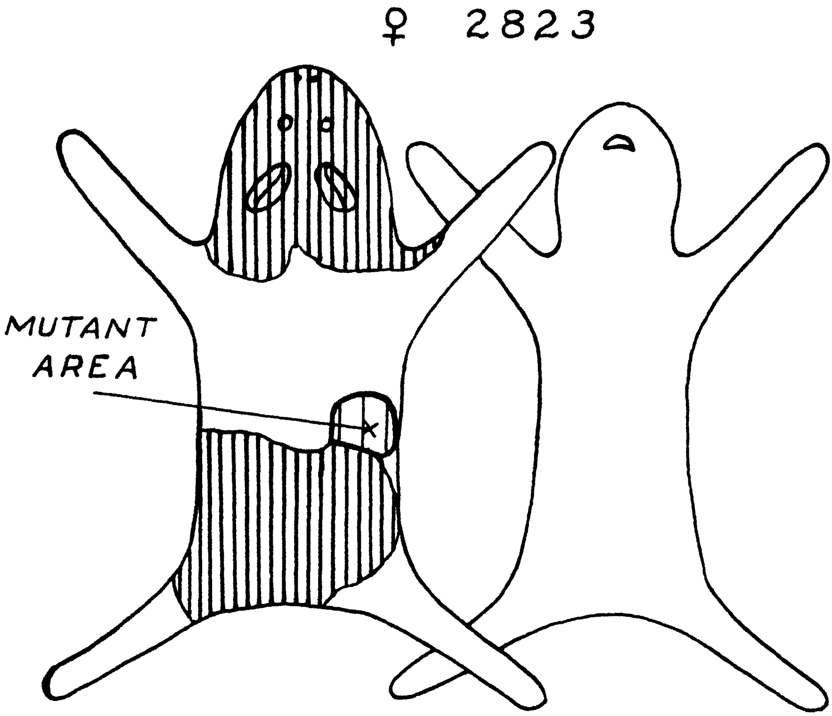


FIGURE 2.
The dorsal coat pattern of ♀ 2823. The heavy vertical lines represent black and the light vertical lines sepia. There was a red spot and another sepia spot on the belly, not shown in the early record. The mutational significance of the sepia spots is very doubtful.

The simplest explanation of this case is that ♂8400 was initially c^dc^d but that a mutation to the dominant allelomorph C occurred in a cell which was ancestral both to part of the soma and part of the germinal epithelium.

It must be confessed, however, that it is difficult to entirely dismiss the thought that one of the intense grandparents somehow transmitted his intensity through the apparently dilute sire or dam. The writers, however, have been unable to devise any explanation along this line which does not raise more difficulties than it solves.

TWO INTENSE-DILUTE MOSAICS

Two intense dilute mosaics may be noted very briefly. One of these (♀2823) was born in one of the inbred families (No. 38) homozygous in all color factors. This guinea pig had four sharply distinct colors in the coat, black, sepia, red and white (figure 2). Incidentally, she had the peculiarity of lacking both eyeballs, a trait which appeared sporadically in family 38. Her 11 progeny were all blacks without any of the abnormal sepia color. A grandson, however, ♂8210, born December 3, 1918, was somewhat like her in his quadri-colored coat. He had a sepia patch on his nose contrasting sharply with the surrounding black as well as with his

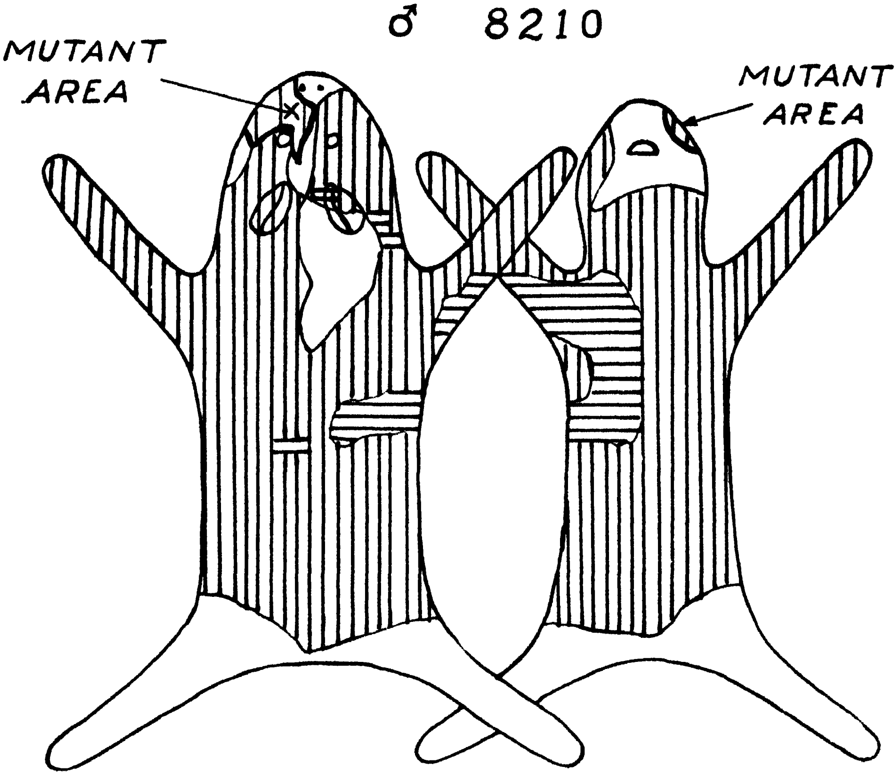


FIGURE 3.
The coat pattern of ♂ 8210 showing black (heavy vertical lines), sepia (light vertical lines) and red (horizontal lines). The mutational significance of the sepia spot is very doubtful.

red spots (figure 3). He came from a mating which brought together four different inbred families (13, 39, 34 and 38) from all of which he could have received only the intensity factor C in the albino series, except for a possibility of receiving albinism, c^a, from family 13. There was no evidence from the numerous progeny of his grandparents and parents or from his own progeny, which included 3 normal blacks from a mating with his dam, and 4 normal blacks from a mating with his sister, that he received or transmitted anything but C in the albino series, or that other factors producing dilution of black were present. A possible explanation is that the sepia spots represented modified brindled spots. Tortoiseshells usually show not only clear-cut black and red areas but brindled areas in which black and red hairs are intermingled. In these areas the black is usually much reduced in intensity. A spot composed exclusively of such hairs, that is, dilute black unassociated with red hairs, would have much the appearance of the observed spots on ♀2823 and ♂8210. If this is the

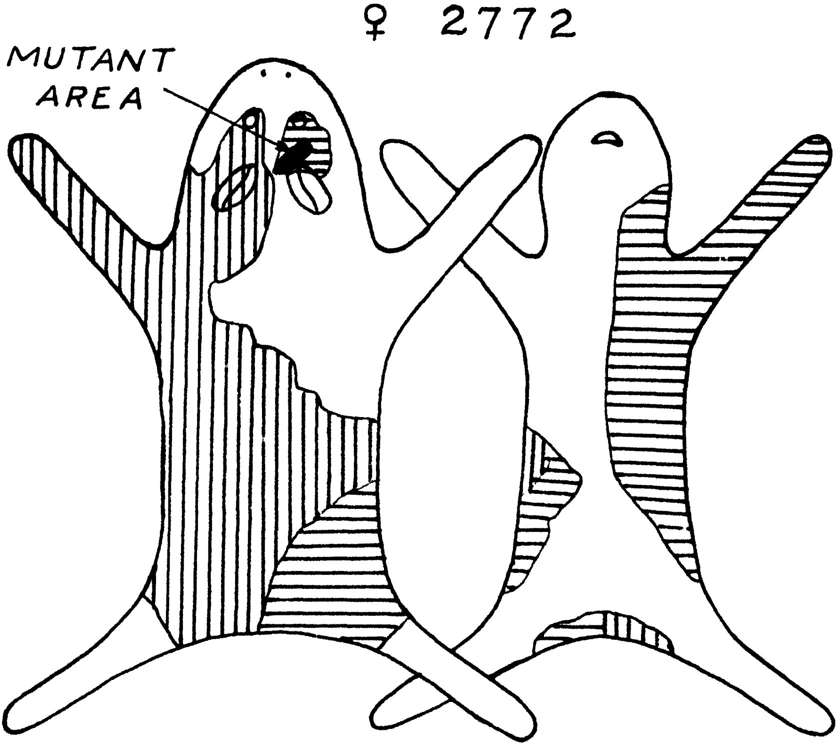


FIGURE 4.
The coat pattern of ♀ 2772 showing golden agouti (vertical lines), black (solid) and red (horizontal lines).

explanation, the mosaic pattern has no mutational significance, being simply a spot due to factor e^p, in which both animals were homozygous. It could be assumed that a peculiar combination of modifying factors gave these spots their unusual appearance.

AN AGOUTI-BLACK MOSAIC ($AA+aa$)

We will next consider the case of an agouti-black-red-white quadricolor, ♀2772, born November 24, 1919, in stock *B*, the control stock for the inbred families. This female had a clear-cut black spot in the fur in front of her right ear, surrounded by red (figure 4). She is the only agouti guinea pig on which the writers have ever noticed as much as a solid black hair. Her parents, grandparents and great-grandparents were all agoutis, except for one red-white bicolored great-grandparent, probably an agouti genetically. Three out of the 16 great-great-grandparents, however, were black. ♀2772 was one of fourteen agoutis produced by her parents. She

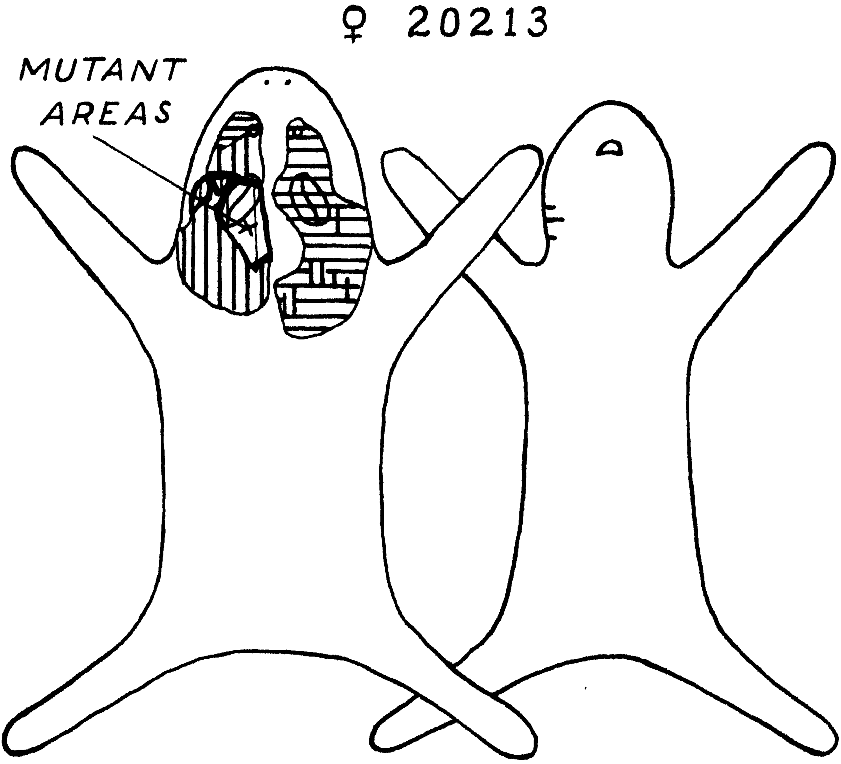


FIGURE 5.
The coat pattern of ♀ 20213 showing golden agouti (heavy vertical lines), cream agouti (light vertical lines) and red (horizontal lines).

herself produced only 14 agoutis in a mating with a black male. Two of her daughters were mated with black males and produced 23 agoutis to 25 blacks, in addition to red-whites and albinos in which the agouti factor can produce no visible effect.

The ancestry and breeding record of ♀2772 make it reasonably certain that she was a homozygous agouti (*AA*) genetically. If the black spot was due to the ordinary recessive black factor (*a*) both genes must have mutated or been lost. The possibility remains, of course, that a mutation to an unknown dominant black occurred in some other locus.

TWO INTENSE-DILUTE MOSAICS ($Ff+ff$)

The next case is that of a golden agouti tricolor, ♀20213, born in the control stock *B*, September 4, 1923. In this case a much broken yellow agouti spot was noted on the left side of the head (figure 5). This spot changed later to a very pale cream agouti, markedly in contrast with the surrounding golden agouti. The black base of these cream agouti hairs was of full intensity however. These effects all indicated the kind of dilution due to factor *f* rather than the dilution of the albino series. The sire was an albino, whose sire came from mating *B*211 which (with its parent matings) is the only known source of factor *f*. The paternal great-granddam of ♀20313, curiously enough, was ♀2772, the agouti-black mosaic discussed above. The dam of ♀20213 was a red-white from golden-agouti tricolor parents and grandparents. The parents produced 23 golden-agouti tricolors, 7 black-red-white, 2 red-white in addition to the mosaic. This result indicates that the dam was *CCFF*, the sire being probably c^ac^aFf.

Female 20213 was herself tested by mating with a black-yellow-white male, known to be Cc^aff from his parentage. She produced 2 golden agoutis. 2 yellow agoutis, 2 black-yellows and 2 albinos. The yellow-agoutis and black-yellows were clearly of the kind due to factor *f* and not to c^k or c^d. It was thus clear that ♀20213 was of constitution Cc^aFf. The mutant spot can be explained as due either to a mutation from *F* to *f* in the ancestral cell, or to loss of the whole chromosome carrying *F*.

The case of ♂23538 is very similar to that just described. This animal, born April 20, 1924, in the control stock *B*, was originally called a golden-agouti tricolor. When three months old a conspicuous cream-agouti spot was observed on the left side (figure 6). As in the previous case, the appearance suggested factor *f*. Guinea pigs of formula *ff* typically show yellow of grades 6 to 8 at birth, but in a few cases there has been very little dilution (grade 9) at this time. This probably accounts for the failure to note the spot earlier. Whatever the color at birth, there always seems to be a fading to pale cream, if not to white, later in life.

The parents were both golden-agouti tricolors. They produced 19 golden agoutis (including ♂23538) and 5 yellow agoutis, proving that both carried a dilution factor. The dam was a granddaughter of an *ff* dilute from mating *B*211, previously spoken of as the source of this factor. The sire traced through three generations to the mating which produced the female in *B*211 and which must thus have transmitted factor *f*. Dilution of the albino series (c^k) was also present on the sire's side, but seems to have been absent from the dam's pedigree. The sire was probably Cc^kFf, the dam $CCFf$. Male 23538 so far has produced 4 intense young from a mating with a black-yellow (*ff*) 4 intense from a meeting with an albino (c^ac^aFF) aside from four intense from a mating with an intense female. In all probability, he is *Ff* although the breeding test has not yet proved it.

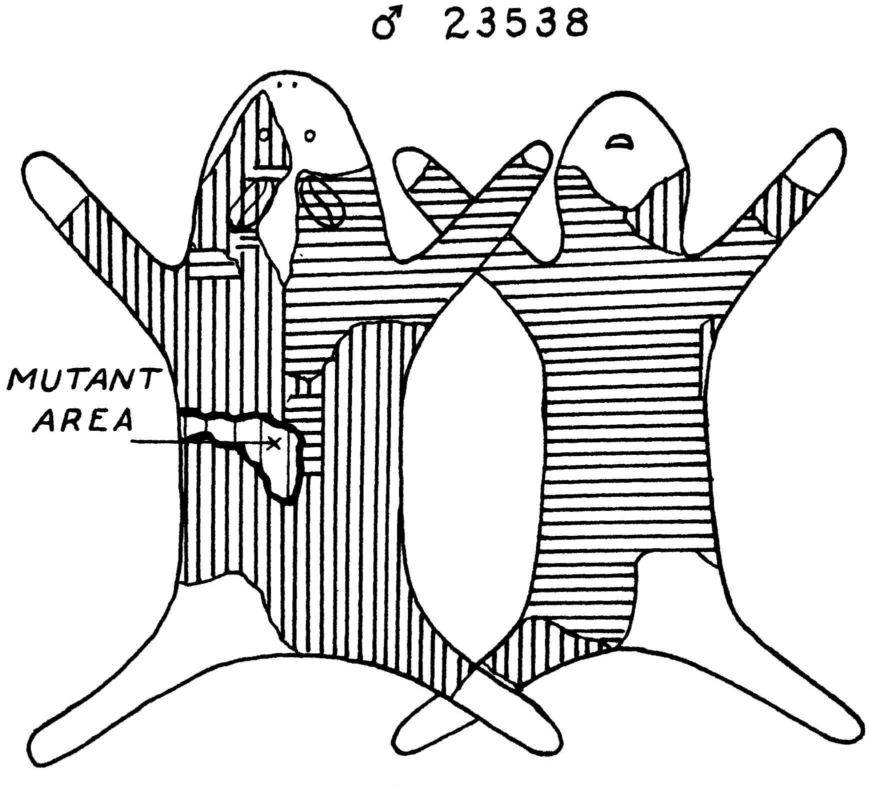


FIGURE 6.
The coat pattern of ♂ 23538, showing golden agouti (heavy vertical lines), cream agouti (light vertical lines) and red (horizontal lines).

The appearance of the cream-agouti spot suggests, as in the preceding case, that either F mutated to f or that the whole chromosome which carries it, was lost in the ancestral cell.

AN INTENSE BROWN-PALE BROWN MOSAIC ($Pp+pp$)

The last case is that of ♀25593, born September 5, 1924. She shows four colors, a typical intense brown and a sharply contrasting pale brown exactly like that due to the pink eye factor p, in addition to the red and white of a tricolor (figure 7). The pale brown covers most of the head and left shoulder. An intense brown spot begins back of the right eye. Although the left eye is entirely surrounded by a pale brown fur and the right eye is more than half surrounded by it, the eyes are both of an intense brown, giving the head a very peculiar appearance to one familiar with guinea pig colors. The retina of the eye has, of course, such a different lineage,

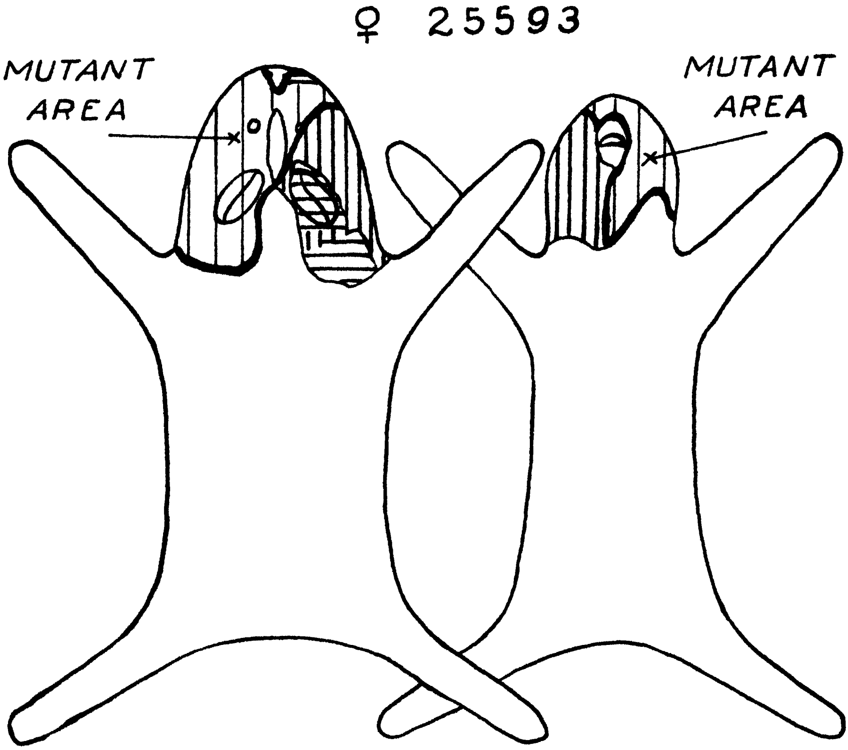


FIGURE 7.
The coat pattern of ♀ 25593 showing intense brown (heavy vertical lines), very pale brown (light vertical lines) and red (horizontal lines).

ontogenetically, from the skin of the head that the lack of agreement requires no explanation except that required for the mosaic pattern as a whole.

This mosaic came from a back-cross involving six color factors, $SsEe^pAaCc^kBbPp \times sse^pe_paac^kc^rbbpp$. The appearance of ♀25593 indicates the constitution $sse^pe^paa\ Cc^{kr}bb\ (Pp+pp)$. The pale brown spots can be explained as due either to a mutation from P to p or to loss of the chromosome carrying P. In a mating with a pink-eyed dilute brown tricolor ($sse^pe^paac^kc^kbbpp$) she has produced 6 brown-eyed young and 2 pink-eyed young, demonstrating that she transmits factor P.

DISCUSSION

Bud mutations and many cases of variegation have been reported in plants in which the abnormal parts have been shown to transmit a corresponding mutant character. These results indicate that mutation may take place at any time in the life history. In animals, we naturally look to Drosophila for the most extensive data. Here also many somatic mutations have been found (BRIDGES 1919; MULLER 1920). BRIDGES finds, however, that most mutations occur as single individuals which he interprets as meaning that mutation is most likely to occur at or very near maturation. He reports a few cases in which a fly has produced a number of mutant offspring, indicating that mutation relatively early in the germ tract had caused the individual to be germinal mosaic. He also reports mutants which appear as somatic mosaics due, presumably, to mutation subsequent to cleavage in a cell ancestral to the germ tract and part of the soma. MULLER (1920) reports similar cases as well as a case of a purely somatic mosaic. MOHR (1923) and MORGAN, BRIDGES and STURTEVANT (1925) report cases in which both soma and germ-plasm were mosaic, as in the mutant guinea pig No. 8400 reported here. MULLER points out that even if mutation is equally likely to occur at all stages of development, most mutations will actually be observed as single individuals, thus taking issue with BRIDGES' interpretation. Gynandromorphs in Drosophila are somatic mosaics which seem ordinarily to be due to dropping out of one of the X chromosomes in cells subsequent to cleavage (MORGAN and BRIDGES 1919). BREITENBECHER has reported numerous dominant purely somatic mutations in the cowpea weevil, Bruchus.

In vertebrates, relatively few such cases have been reported where there was any knowledge of the genetic situation. Three cases described by CASTLE and by CASTLE and PHILLIPS may be mentioned. One of these (CASTLE 1912) concerned a guinea pig which was close to an albino in appearance but had small areas of sepia on the right side of the head, the hips and dark streaks in the iris of each eye. The parents were both colored but one at least was known to transmit albinism. CASTLE (1922) interprets the case as probably a heterozygote, c^dc^a, in which factor c^d was lost from a cell at a very early stage by non-disjunction. He explains similarly a tricolor rat, gray, yellow and white, known to be heterozygous for pink-eyed yellow (*Pp*). This rat had many separate yellow spots. Breeding tests carried to F_2 gave no indication that he transmitted any new factors. The third case occurred in CASTLE and PHILLIPS' experiments on changing the hooded pattern of rats by selection. In the tenth generation of plus selection an ordinary "plus" rat sired two mutants from different females and presumably many normal plus young. These mutants, intermediate between the plus hooded stock and self color, proved to be heterozygous for a new third allelomorph of the hooding factor, dominant over the latter but recessive to self color. This dominant mutation occurred apparently in at least two germ cells of the sire who was thus a germinal mosaic.

Returning to the guinea pigs described in the present paper no one explanation seems to fit the 7 mosaics discussed above. In the last three cases the mutant animals were certainly or very probably heterozygous in the factor affected (*Ff* two cases, *Pp* one case). The loss of the chromosome carrying the dominant factor in one of the somatic divisions seems to be the most plausible explanation, with mutation to the recessive as a possible alternative. The case of the black spot on a homozygous agouti (*AA*) requires the loss of two chromosomes (not necessarily simultaneously) or a double mutation or a mutation to an unknown dominant black. The two black-sepia mosaics were similarly homozygous in all known factors affecting intensity of color, but the possibility that the sepia was merely an aberrant effect of the tortoiseshell factor e^p, in which they were homozygous, makes them of little value.

The case of ♂8400 is the most remarkable one observed. His record may be summarized as follows:

(1) He was a dilute-intense mosaic somatically.

(2) He was produced by extensively tested dilute (recessive) parents each of which, however, had one intense parent.

(3) He transmitted the dominant mutant factor for intensity.

(4) He was a mosaic germinally. His germ cells were about one-third C, two-thirds c^d as indicated by a ratio of 79:149 in backcrosses.

(5) The proportion of *C* gametes seems to have varied from time to time.

(6) Both intense and dilute progeny bred normally.

These results can all be explained by the hypothesis that he was c^dc^d initially but that one c^d mutated to C in a cell ancestral to part of the soma and also to part of the germinal epithelium and that the activity of different parts of the latter varied from time to time. The possibility of a rather complicated explanation involving aberrant chromosome behavior is not perhaps entirely ruled out.

The appearance of these mosaics throws some light on a number of questions. Factors which affect coat color may be conceived either as producing their effects by local action or by means of substances carried in the blood. The differently colored spots of a tricolor are quite obviously determined locally. Even here, however, it is not necessary to suppose that the piebald and tortoiseshell factors act locally. Sex is a factor which modifies the extent of colored spots in piebalds and of black spots in tortoiseshells but which need not be thought of as acting locally. The relatively uniform color differences due to factors a, c^d, f, b, and p, it would seem, might well be constitutional rather than local. Nevertheless, the cases of persistent somatic mosaics, in all probability due to mutation or loss of certain of these factors in particular somatic cells, indicate that the effects are produced locally or, in other words, that the pigment produced by a particular cell depends, in the main, on the genes in its own nucleus. The evidence is reasonably satisfactory for factors c^a, f and p, less so for a. No case apparently involving b has yet been found.

Another point of interest is the shape of the mutant spots. If each is due to a single mutation or chromosome loss, it appears that a single cell may give rise to rather widely scattered descendants. This seems to have been the case with CASTLE'S tricolor rat mosaic. The mutant areas of ♂8400 are widely scattered, as may be seen from the figure. The wide separation of the head and shoulder spots of red-agouti on ♂8400 from the rump spot is especially important in view of the completeness of this record, including evidence that his germinal epithelium was also mosaic. The cream-agouti spots in ♀20213 and ♂23538 are very irregular in shape. In ♀20213 the cream-agouti is all on the left side of the head, but is broken into at least three portions, separated by red-agouti. In ♂ 23538, the spot runs irregularly from the mid-dorsal line to the belly down the middle of the side. In both of these cases, the shape is more irregular than is characteristic of either the colored or white spots of a piebald. Similar spots could perhaps be found on a tortoiseshell. The pale brown area of ♀25593 is compact enough except that it is cut by the white nose streak. The genetic condition of the mutant area doubtless is present in this streak. Similarly, ♀2823 had two separate sepia spots but as these were separated merely by white, there is not necessarily any genetic discontinuity. Male 8210 and ♀2772 each had merely one compact mutant area.

Another curious fact is the relationship between certain of the mutants. Female 2823 (black-sepia) was the granddam of ♂8210, also black-sepia. Female 2772 agouti-black ($AA+aa$?) was the great-granddam of ♀20213 (red-cream-agouti) ($Ff+ff$) agouti and ♂23538 ($Ff+ff$) was related fairly closely to both of these. The case of the two black-sepias may, as noted in the text, involve merely inheritance of certain peculiar modifiers and not belong in the class of mosaics discussed here. The other cases may indicate an hereditary tendency toward germinal instability. Little (1916) notes as possibly due to such a cause the occurrence of mutation from wild gray to brown-agouti (B to b) and from ordinary wild gray to white-bellied gray (A to A^1) in the descendants of a certain wild-gray mouse. The relationships here noted are rather closer than would be expected by chance in the large stock of guinea pigs of many distinct strains maintained by the Bureau of Animal Industry

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THE GENETICS OF *LYCOPERSICUM ESCULENTUM* MILL
1. THE TRISOMIC INHERITANCE OF "DWARF"[1]

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Received November 23, 1925

The occurrence of triploidy in the tomato has recently been reported (LESLEY 1925). In 1923 one of two triploid plants of Dwarf Aristocrat, a dwarf variety with scarlet fruit, was crossed with Globe, a diploid with standard habit and crimson fruit. The dwarf habit is a simple recessive to standard, and the yellow skin of scarlet fruit is a simple dominant to the colorless skin of crimson. An F_1 of three plants was grown, all of which were different in appearance from the F_1 diploid Dwarf Aristocrat × Globe and also from one another. Two of them were found to have 26 chromosomes or 2 more than the diploid (somatic) number; as was to be expected from their origin, these plants were double trisomics. The third plant developed two cotyledons and one pair of first leaves but no bud. Its chromosome number was not determined.

The more vigorous of the 26-chromosome plants, no. 23. 123.1, was a standard with scarlet fruit. It was less hairy than the diploid F_1, the leaves were decidedly thicker and more leathery in texture and their surface more rugose. The margin of the leaflets had fewer deep indentations and was more undulate. The lateral leaflets were narrower than in the diploid, but the terminal one was broader than the laterals and often somewhat twisted. The sepals were shorter and the petals somewhat deeper yellow, narrower at the base and blunter. The stamens were more slender, paler yellow and often incompletely syngynesious. The pollen was less in amount and contained more empty grains. The stigma, unlike that of the diploid, was usually below the apex of the staminal cone. One flower was entirely without a gynoecium. Under field conditions the plant was unfruitful unless artificially pollinated. The fruit was somewhat less regular in shape than that of the diploid F_1, and the seeds were relatively few.

This plant gave rise to an F_2 consisting of 41 plants, of which 3 were found to be simple trisomic, while the remainder were probably diploid. The diploids consisted of 22 standards and 16 dwarfs, a departure from the disomic 3:1 ratio towards the trisomic ratio of 5:4 which would be expected to result if one of the two extra chromosomes of the parent were that containing the locus of the dwarf-standard pair of genes. Since the triploid P_1 was dwarf, the F_1 would contain two dwarf and one standard-bearing chromosomes or the genes *Ddd*. With regard to fruit color 27 plants were scarlet and 9 crimson, which corresponds exactly to the disomic ratio of 3:1.

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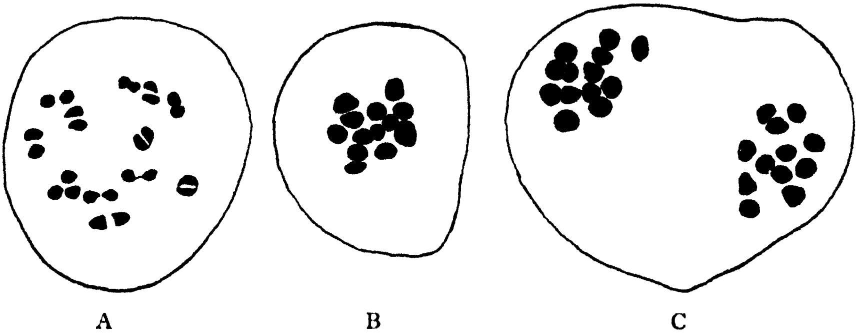
[1] Paper No. 140, UNIVERSITY of CALIFORNIA, Graduate School of Tropical Agriculture and CITRUS EXPERIMENT STATION, Riverside, California.

One of the three simple trisomic plants, no. 24.094.32, was a standard. The figure was drawn with a camera lucida from smears made by Belling's iron-acetocarmine method. Although this plant was nearer its diploid sibs in hairiness and in color of corolla, it was decidedly slower-growing and later in flowering and in general much more nearly resembled its 26–chromosome parent. The foliage was similar in shape and texture, the sepals short and the stigma retruded. The style and stigma were perhaps more often misshapen than in the 26-chromosome F_1 plant. There was a similar tendency to pollen sterility and it set no fruit in the field without artificial pollination. The ripe fruit held tenaciously at the articulation. Apparently one of the extra chromosomes present in the 26-chromosome combination of plant 23.123.1 produced comparatively little external effect. It should be noted that both the F_1 plant 23.123.1 and the F_2 plant 24.094.32 although standard in general habit, showed certain dwarf-like characters, especially in the texture and rugosity of the leaves and in the short sepals and style.



Chromosomes in pollen-mother-cells of simple trisomic tomato, 24.094.32. Stained in acetocarmine.

A. Diakinesis. Eleven bivalents and one trivalent. ×2450.
B. Metaphase of heterotype division. Twelve bivalents and one univalent at periphery and less deeply stained. ×3050.
C. Metaphase of homotype division. Extra chromosome present in one plate undivided. ×4000

In the selfed family from the simple trisomic 24.094.32, the germination was 94 percent and nearly all the seedlings survived. Among the diploid progeny there were 17 standard and 20 dwarf plants, which again accords better with the 5:4 ratio expected in trisomic inheritance than with the disomic ratio of 3:1. The simple trisomic plants, expecially the standards, were clearly recognizable by their close resemblance to the parent 24.094.32, and 6 of them were confirmed as such by chromosome counts. They consisted of 8 standard and 3 dwarf plants, a ratio which approaches the expected trisomic ratio of 7:2.

In a cross between Dwarf Champion and the same simple trisomic plant 24.094.32, the expected ratio among diploids was 1 standard to 2 dwarfs. The observed result was 14 standard and 29 dwarfs. The germination was excellent and every seedling survived but there were no trisomic progeny. A similar tendency to non-transmission of certain extra chromosomes through the pollen was found by BLAKESLEE (1921) in Datura, by DEVRIES and BOEDIJN (1923) in Oenothera, by CLAUSEN and GOODSPEED (1924) in Nicotiana and by FROST and MANN (1924) in Matthiola. Assuming this to be the case here, the data from selfing would indicate a transmission of the extra chromosome through about 23 percent of the eggs.

SUMMARY

Two 26-chromosome plants have been found in the F_1 progeny of triploid Dwarf Aristocrat × Globe. Selfing of one of these produced a simple trisomic plant of the standard type which closely resembled its double trisomic parent and differed in several respects from related diploids. It was slower-growing and showed certain dwarf-like characters. Breeding results show that the extra chromosome present in the simple trisomic was that containing the locus of the standard-dwarf pair of genes. The extra chromosome was not transmitted through the pollen but was present in about 23 percent of the progeny from selfing.

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INHERITANCE OF PLUMAGE COLOR IN THE RHODE ISLAND RED BREED OF DOMESTIC FOWL[1,2]

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INTRODUCTION

The color pattern in the Rhode Island Red is known to be highly variable. In an ordinary flock coming from "standard" ancestry may be seen birds of such a pale shade of red as to be almost buff, birds of about the color tone of English red, mahogany red birds, bay birds, etc. Auburn appears to be a rather common shade of color in the flock studied.

The amount of melanic pigment present fluctuates widely as shown by varying degrees of stippling, mottling, and ticking as well as by the occurrence of red feathers spangled with black. The variability in color pattern for this particular breed of fowl suggests that the so-called "standard" or ideal color pattern is of rather complex nature.

"Standard" color according to the 1923 edition of the Standard of Perfection is rich brilliant red and rich even red, except for the presence of black in the flight feathers and part of the flight coverts and tail in both sexes and in the lower neck feathers of the female. The shade of red preferred depends somewhat upon individual opinion, but at any

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rate, bay is generally in greatest favor. The ideal specimen is apparently one showing the same shade of red over all sections of the body. In the study to be presented below, "standard" color is applied only to those birds closely approaching bay, in the surface plumage and carrying black in flight feathers and tail of both sexes and black in neck feathers of the female. The under-color will be specified as black or blue, smutty or light, if not red.

BITTENBENDER (1922) believes that a bar of slate or black pigment in the back feathers of either males or females used as breeders is necessary to secure rich, red plumage color. He states that only one parent should carry this pigment bar in mating for "standard" color. He emphasizes the importance of using both male and female breeders of the same color shade.

GOODALE (1910) has shown that the Brown Leghorn color pattern is sex-linked. He (1911) also refers to some F_2 birds in a cross between Brown Leghorn and Buff Plymouth Rocks as showing the Rhode Island Red color. Further details concerning this particular color have not been published.

AGAR (1924) makes reference to a pigment called "chestnut" which he discovered in crosses between Barred Rocks and Rhode Island Reds. This pigment is brown in color and is independent of the gene for gold (s) and apparently not sex-linked in its inheritance.

LLOYD-JONES (1915) found all pigment granules in red pigeons' feathers to be chestnut in color and globular in shape. His observations with those of LADEBECK (1922) would suggest that red in the pigeon and in the Rhode Island Red are similar in both morphology and color tone of pigment granules.

According to LADEBECK (1922), all red feathers from Rhode Island Reds contain reddish-brown to reddish-yellow pigment globules of melanic nature. The black feathers carry black pigment granules, some rod-shaped and some eliptical. In addition, the dark feathers carry pigment globules of varying sizes. The presence of both rod-shaped and eliptical pigment granules in the dark feathers appears to be characteristic of the Rhode Island Red.

DUNN (1922) has suggested that Rhode Island Reds carry the recessive gene e^m which restricts melanic pigment to the neck, wings, and tail. The normal allelomorph E^m is therefore not present in Rhode Island Reds so that the xanthic factor for red is not obscured. This variety therefore carries a definite pattern factor which restricts black pigment to neck, wings, and tail as called for in the standard color.

Lippincott (1923) has demonstrated that Rhode Island Reds carry a gene E which extends black pigment throughout the plumage. When genes E^m and E are brought together in the same individual, blue plumage results. In order to account for blue rather than black in such cases, it is necessary to assume that some restriction factor such as Lippincott's R is present so that the melanic pigment is so distributed as to give blue rather than black feathers.

STOCK USED IN THIS EXPERIMENT

The birds used in this experiment consisted of recessive whites and pure-bred Rhode Island Reds. The recessive white stock came from a White Plymouth Rock foundation crossed on Brown Leghorns and later bred back to Brown Leghorns and finally to Rhode Island Reds from which extracted whites came in later generations. Part of the foundation stock was developed by Dr. H. D. Goodale. Breeding tests showed that these whites were free from such cryptomeres as barring, spangling, or silver. Such whites always bred as true recessives.

The Rhode Island Reds used consisted of two general classes, namely, a group with wide variability in color pattern that came from a "standard" foundation, that had been bred for fecundity for a period of ten years without attention to color pattern and two "standard" color males purchased from a prominent breeder. All Rhode Island Reds were pure-bred in the ordinary use of the term.

METHODS OF EXPERIMENTATION

In order to determine the genetic constitution of the Rhode Island Red for color pattern, it was necessary to cross different phenotypes on recessive whites and later to ascertain the genetic constitution of phenotypes resulting. It will be observed that Rhode Island Reds, crossed on recessive whites, give progeny that may be grouped into several general phenotypes. The plan was to test these different phenotypes by mating *inter se*. The experimental crossing began in 1923 and was continued in 1924 and 1925. All resulting progeny were described in adult plumage or at about 150 days old. Feather samples were taken at this time from five body regions. Feather samples were plucked from hackle, mid-back, main tail, breast, and body fluff. No attempt was made to record the juvenile plumage. All birds were pedigree hatched and were given a permanent wing and a permanent leg band each bearing the same number.

EXPERIMENTAL RESULTS

Proposed Theory

A study of the color pattern in progeny resulting from crosses made on three successive years suggests the following concerning the factor complex in color in Rhode Island Reds:

Gene B is an autosomal gene for reddish-brown pigment which distinguishes Rhode Island Reds from the gold of the Brown Leghorn or the buff found in the Orpington, Buff Leghorn, etc. Buffs have been shown by Dunn (1922) to be e^me^mss but the relation between buff and gold has not been determined so far as I am aware. Gene B with either L or E or both always results in some shade of red on the surface. If present with factor L only the red pigment extends to the under color as well. If B is present, with factor E only, there will be brown stippling in the females. B in the presence of both L and E gives smutty under-color but a red surface. Factor B alone gives white, the presence of L and E in the absence of B gives the Brown Leghorn or Jungle fowl pattern, and either L or E alone gives white.

Gene L is a sex-linked gene for color pattern and gold color. Its presence makes possible the appearance of distinct color areas on wings, breast, etc., as in the Jungle fowl when the necessary pigment and extension factors are present.

Gene E is an autosomal gene for the extension of melanic pigment throughout the feathers such as Lippincott has pointed out in his studies on blue color.

Gene R, Lippincott's factor for restriction of pigment within the feather and for the shape of pigment granule, is probably present in those birds showing blue smut in under-color.

Gene e^m is also present as Dunn has previously pointed out.

Gene C' of Hadley (1913) is also present.

The chief concern in this report is with genes B, L, and E as these three or their allelomorphs in the various combinations give the distinct phenotypes that make up Rhode Island Red color pattern. Attention is called to the fact that this study deals with distinct color phenotypes and has not been carried far enough to discover possible variations in shade of red that might result from multiple factors. Further experimental tests now being carried on should further elucidate this question.

The different phenotypes studied in this experiment together with their probable genetic composition are listed below for each sex.

COLOR ANALYSIS-PHENOTYPES AND GENOTYPES

Males

Phenotypes	Genotypes
Standard Red—No Smut	*BBLLee* *BBLlee*
Standard Red—No Smut	*BBllEE* *BBllEe*
Standard Red—Smutty	*BBLLEE* *BBLLEe* *BBLlEE* *BBLlEe*
Light Red—No Smut	*BbLLee* *BbLlee*
Medium Red—No Smut—Stippled	*BbllEE* *BbllEe*
Medium Red—Smutty	*BbLLEE* *BbLLEe* *BbLlEE* *BbLlEe*
Leghorn Color—Smutty Black Breast	*bbLLEE* *bbLLEe* *bbLlEE* *bbLlEe*
White	*BBllee* piled *Bbllee* piled *bbLLee* *bbLlee* *bbllEE* *bbllEe* *bbllee*

Females

Phenotypes	Genotypes
Standard Red—No Smut	*BBLoee*
Standard Red—No Smut—Stippled	*BBloEE* *BBloEe*
Standard Red—Smutty	*BBLoEE* *BBLoEe* stippled
Light Red—No Smut	*BbLoee*
Medium Red—No Smut—Stippled	*BbloEE* *BbloEe*

Medium Red Smutty	*BbLoEE* *BbLoEe* **stippled**
Leghorn color—Smutty—Stippled Salmon Color	*bbLoEE* *bbLoEe*
White	*BBloee* piled *Bbloee* piled *bbLoee* *bbloEE* *bbloEe* *bbloee*

Experimental Matings—1923

A series of eight matings gave progeny in the spring of 1923 but unfortunately only two resulted in families of sufficient size for genetic study. The total number of progeny described was thirty-one. The probable genetic formulae of the parents with the actual and expected phenotypes of progeny are given below:

Table 1

Piled white male C 4421 (*bbLlee*)×*Red, stippled, smutty female B* 9864 (*BbLoEe*).

CHARACTER	PULLETS		COCKERELS	
	Actual	Expected	Actual	Expected
Smutty	2	1	5	3.5
Non-smutty	0	1	1	1.75
White	2	2	1	1.75
Standard red	0	0	2	0
Medium or light red	2	2	4	5.25
White	2	2	1	1.75
Stippled	2	1.5		
Non-stippled	0	.5		
White	2	2		

Table 2

Piled white male C 4421×*White female C* 3369

CHARACTER	PULLETS		COCKERELS	
	Actual	Expected	Actual	Expected
White	4	4	2	2

Experimental Matings—1924

In the spring of 1924 the total number of matings giving progeny was nine, with 54 cockerels and pullets described. There were five matings that gave sufficient numbers of adult progeny for study. The probable genetic formulae of the parents together with the phenotypes of their progeny and the expected ratios are given below:

TABLE 3

Standard smutty Rhode Island Red male C 75450 (*BBLLEe*)×*White female C* 3369 (*bbloEe*)

CHARACTER	PULLETS		COCKERELS	
	Actual	Expected	Actual	Expected
Smutty	3	2.25	1	2.25
Non-smutty	0	.75	2	.75
White	0	0	0	0
Medium red	3	3	3	3
Light red	0	0	0	0
White	0	0	0	0
Stippled	2	1.50		
Non-stippled	1	1.50		
White	0	0		

TABLE 4

White male C 9551 (*bbLlee*)×*Dark Rhode Island Red female C* 4518 (*BbLoEE*)

CHARACTER	PULLETS		COCKERELS	
	Actual	Expected	Actual	Expected
Smutty	3	2	1	3
Non-smutty	1	1	2	0
White	0	1	0	0
Medium red	2	2	3	1.5
Light red	2	1	0	1.5
White	0	1	0	0
Stippled	1	3		
Non-stippled	3	0		
White	0	1		

TABLE 5

White Male C 9551 (*bbLlee*)×*Very dark Rhode Island Red female C* 5701 (*BbLoEe*)

CHARACTER	PULLETS		COCKERELS	
	Actual	Expected	Actual	Expected
Smutty	4	2.25	3	2.50
Non-Smutty	5	4.50	2	1.25
White	0	2.25	0	1.25
Medium red	9	6.75	3	2.50
Light Red	0	0	2	1.25
White	0	2.25	0	1.25
Stippled	7	4.50		
Non-stippled	2	2.25		
White	0	2.25		

TABLE 6

Medium red smutty male C 9066 (*BbLLEe*)×*White female C* 8737 (*bbloce*)

CHARACTER	PULLETS		COCKERELS	
	Actual	Expected	Actual	Expected
Smutty	3	3	2	1
Non-smutty	1	1.5	0	.5
White	2	1.5	0	.5
Medium red	3	3	2	1
Light red	1	1.5	0	.5
White	2	1.5	0	.5
Stippled	4	3		
Non-stippled	0	1.5		
White	2	1.5		

TABLE 7

Black body, smutty male C 8619 (*bbLlEe*)×*White female C* 8473 (*bbloce*)

CHARACTER	PULLETS		COCKERELS	
	Actual	Expected	Actual	Expected
Smutty	1	.75	0	.75
Non-smutty	0	0	0	0
White	2	2.25	3	2.25
Standard red	0	0	0	0
Light red	1	.75	0	.75
White	2	2.25	3	2.25
Stippled	1	.75		
Non-stippled	0	0		
White	2	2.25		

Experimental Matings—1925

In the spring of 1925, twenty matings were made in this experiment. Of these matings sixteen gave progeny in fair numbers and are reported below. The total number of progeny described from the twenty matings is 88. A description of the parentage is given for the matings reported together with the actual and expected ratios of progeny.

TABLE 8

Medium red slightly smutty male E 1125 (*BbLlEe*)×*Light red, stippled blue smutty female E* 131 (*bbLoEe*)

CHARACTER	PULLETS		COCKERELS	
	Actual	Expected	Expected	
Smutty	1	1.13	4	3.75
Non-smutty	1	.56	0	.63
White	1	1.31	1	.62

TABLE 8 (continued)

CHARACTER	PULLETS		COCKERELS	
	Actual	Expected	Actual	Expected
Standard red	0	0	0	0
Medium and light red	2	1.69	4	4.37
White	1	1.31	1	.63
Stippled	2	1.50		
Non-stippled	0	.19		
White	1	1.31		

TABLE 9

Male E 1125 (BbLlEe) × *Dark red, stippled, black smutty female E 132 (BbLoEe)*

CHARACTER	PULLETS		COCKERELS	
	Actual	Expected	Actual	Expected
Smutty	0	1.88	2	2.25
Non-smutty	2	1.88	0	.55
White	3	1.24	1	.20
Standard red	2	1.09	1	.75
Medium red	0	2.66	1	2.05
White	3	1.25	1	.20
Stippled	2	2.81		
Non-stippled	0	.94		
White	3	1.25		

TABLE 10

Male E 1125 (BbLlEe) × *Medium red, black smutty female E 134 (BbLoEE)*

CHARACTER	PULLETS		COCKERELS	
	Actual	Expected	Actual	Expected
Smutty	5	2.50	2	2
Non-smutty	0	1.87	0	0
White	0	.63	0	0
Standard red	3	1.25	2	.50
Medium red	2	3.12	0	1.50
White	0	.63	0	0
Stippled	3	3.12		
Non-stippled	2	1.25		
White	0	.63		

TABLE 11

Male E 1125 (*BbLlEe*)×*Standard red, smutty female E* 480 (*BbLoEe*)

CHARACTER	PULLETS		COCKERELS	
	Actual	Expected	Actual	Expected
Smutty	1	.75		
Non-smutty	0	.75		
White	1	.50		
Standard red	1	.75		
Medium red	0	.75		
White	1	.50		
Stippled	1	1.12		
Non-stippled	0	.38		
White	1	.50		

TABLE 12

Male E 1125 (*BbLlEe*)×*Standard red, non-smutty female E* 481 (*BBLoee*)

CHARACTER	PULLETS		COCKERELS	
	Actual	Expected	Actual	Expected
Smutty			2	1.0
Non-smutty			0	1.0
White			0	0
Standard			2	1
Medium			0	1
White			0	0

TABLE 13

Male E 1125 (*BbLlEc*)×*Light red, light under-color non-smutty female*
E 482 (*BbLoee*)

CHARACTER	PULLETS		COCKERELS	
	Actual	Expected	Actual	Expected
Smutty	0	.26	1	2
Non-smutty	0	.37	1	1.50
White	1	.37	2	.50
Standard red	0	.19	2	1
Medium red	0	.44	0	2.50
White	1	.37	2	.50
Stippled	0	.44		
Non-stippled	0	.19		
White	1	.37		

TABLE 14

Standard color non-smutty male E 485 (*BbLlee*)×*Medium red stippled non-smutty female E* 135 (*BBLoEe*)

CHARACTER	PULLETS		COCKERELS	
	Actual	Expected	Actual	Expected
Smutty	0	.25	1	.75
Non-smutty	1	.50	2	1.50
White	0	.25	0	.75
Standard red	1	.37	1	1.12
Medium or light red	0	.37	2	1.12
White	0	.26	0	.76
Stippled	1	.50		
Non-stippled	0	.25		
White	0	.25		

TABLE 15

Male E 485 (*BbLlee*)×*Medium red, stippled blue smutty female E* 136 (*BbLoEe*)

CHARACTER	PULLETS		COCKERELS	
	Actual	Expected	Actual	Expected
Smutty	0	.75	2	2 25
Non-smutty	1	.75	0	.56
White	1	.50	1	.19
Standard red	0	.44	1	.75
Medium red	1	1.06	1	2.06
White	1	.50	1	.19
Stippled	1	1.13		
Non-stippled	0	.37		
White	1	.50		

TABLE 16

Male E 485 (*BbLlee*)×*Medium red slight blue smut non-strippled female E* 407 (*BbLoEe*)

CHARACTER	PULLETS		COCKERELS	
	Actual	Expected	Actual	Expected
Smutty	1	.13	1	.88
Non-smutty	0	.37	0	.62
White	0	.50	1	.50
Standard red	0	.13	1	.50
Medium red	1	.37	0	1.00
White	0	.50	1	.50
Stippled	0	0		
Non-stippled	1	.37		
White	0	.63		

TABLE 17

White male E 352 (bbLlee)×Standard color Rhode Island Red female C 7805 (BbLoEE)

CHARACTER	PULLETS Actual	PULLETS Expected	COCKERELS Actual	COCKERELS Expected
Smutty	5	3	4	4
Non-smutty	1	1.5	0	0
White	0	1.5	0	0
Medium red	2	1.5	2	2
Light red	4	3	2	2
White	0	1.5	0	0
Stippled	3	3		
Non-stippled	3	1.5		
White	0	1.5		

TABLE 18

Male E 352 (bbLlee)×Pale color Rhode Island Red female C 9440 (BbloEe)

CHARACTER	PULLETS Actual	PULLETS Expected	COCKERELS Actual	COCKERELS Expected
Smutty	2	.5		
Non-smutty	0	.5		
White	0	1.0		
Standard red	0	0		
Light red	2	1		
White	0	1		
Stippled	2	1		
Non-stippled	0	0		
White	0	1		

TABLE 19

Male E 352 (bbLlee)×Pale colored Rhode Island Red female C 9483 (BbloEe)

CHARACTER	PULLETS Actual	PULLETS Expected	COCKERELS Actual	COCKERELS Expected
Smutty	1	.25	1	.50
Non-smutty	0	.25	1	.50
White	0	50	0	1.00
Standard red	0	0	0	0
Medium or light red	1	50	2	1
White	0	50	0	1
Stippled	1	.37		
Non-stippled	0	12		
White	0	51		

TABLE 20

Spangled smutty male C 8619 (bbLlEc) × Pure white female C 8473 (bbloec)

CHARACTER	PULLETS		COCKERELS	
	Actual	Expected	Actual	Expected
Smutty	1	1.25	2	1.75
Non-smutty	0	0	0	0
White	4	3.75	5	5.25
Standard red	0	0	0	0
Medium or light red	1	1.25	2	1.75
White	4	3.75	5	5.25
Stippled	1	1.25		
Non-stippled	0	0		
White	4	3.75		

TABLE 21

Male C 8619 (bbLlEc) × Salmon, blue smutty, stippled female E 225 (bbLoEc)

CHARACTER	PULLETS		COCKERELS	
	Actual	Expected	Actual	Expected
Smutty	1	.38	1	1.50
Non-smutty	0	0	0	0
White	0	.62	1	.50
Standard red	0	0	0	0
Light red	1	.38	1	1.50
White	0	.62	1	.50
Stippled	1	.12		
Non-stippled	0	.25		
White	0	.63		

TABLE 22

Male C 8619 (bbLlEc) × White hens E 353 and E 801 (bbloec)

CHARACTER	PULLETS		COCKERELS	
	Actual	Expected	Actual	Expected
Smutty	0	.75	0	.25
Non-smutty	0	0	0	0
White	3	2.25	1	.75
Standard red	0	0	0	0
Light red	0	.75	0	.25
White	3	2.25	1	.75
Stippled	0	0		
Non-stippled	0	.75		
White	3	2.25		

TABLE 23

Male C 8619 (*bbLlEe*)×*Female C* 8473 (*bbloee*)

CHARACTER	PULLETS		COCKERELS	
	Actual	Expected	Actual	Expected
Smutty	2	2	2	2.5
Non-smutty	0	0	0	0
White	6	6	8	7.5
Standard red	0	0	0	0
Medium red	2	2	2	2.5
White	6	6	8	7.5
Stippled	2	2		
Non-stippled	0	0		
White	6	6		

DISCUSSION OF RESULTS

Breeding tests demonstrate conclusively that color pattern in Rhode Island Reds is rather complex genetically. These tests help to explain why there is such a diversity in color pattern even in those flocks bred for "standard" color. In recent years, the trend of color shade has been from mahogany red to bay or even brown in some cases. There has been considerable difficulty in classifying birds as to medium or "standard" color. Such a classification is at best an arbitrary one and such discrepancies as are shown in the ratios are to be expected. This demand for deeper pigmentation has caused breeders to select as breeding stock those birds with the most intense black pigment in wings and tail and dark brown in body color. The use of some breeding stock carrying smut in under-color of back has also been common. Birds exhibiting a medium shade of red in surface plumage will actually appear darker in general plumage color if they carry black stippling in some degree or if they carry dark smut in under-color. Birds carrying black pigment in any part of the plumage, except the wings and tail or the neck of females, are considered "off color" but probably more desirable than birds deficient in black, and showing a light shade of red. The actual demand is for a deeper intensification of brown pigment granules and not for more black in the plumage.

The interaction of genetic factors involved in so far as they affect the color pattern really governs progress. These studies based on 173 progeny have strongly suggested that the true "standard" color Rhode Island Red male represents but four genotypes, namely, *BBLLee*, *BBLlee*, *BBllEE*, and *BBllEe*. That the true "standard" color female represents but three genotypes, namely, *BBLoee*, *BBLoEE*, and *BBloEe*. The undesirable smutty color makes its appearance whenever genes *B*, *L*, and *E* come together in the same zygote. Also, when genes *L* and *E* are present in the same zygote, the smutty under-color appears. Smutty under-color does not appear when genes *B* and *E* are brought together because *E* acts only in the presence of the pattern factor *L*. Males of the genetic formula *bbLLEE* will be of the same color as the Brown Leghorn or the Jungle fowl. Females of the genetic formula *bbLoEE* will be salmon in the body because of the presence of gold and smut.

Stippling has been observed to be of two general kinds, black and brown. Black stippling occurs most frequently on wings and breast in both males and females. Brown stippling appears to be confined to the female sex only. It may be restricted to the back and wings or may extend all over the body. Black stippled birds are probably those carrying genes *B*, *L*, and *E* while brown stippled birds appear to carry only genes *L* and *E*. The male phenotype that corresponds to the brown stippled female is apparently the black-breasted, light, smutty bodied male similar in color pattern to the Brown Leghorn male.

White birds are of several genotypes. They may carry any one of the three genes *B*, *L*, and *E* or may lack all and be true recessives. Birds of the composition *BBllee* are white because factor *B* acts only in the presence of *L* or *E*, *bbLLee* birds are white because the extension factor *E* is wanting and *bbllEE* birds are white because there can be no extension of pigment without the pattern factor*L*. True recessives are *bbllee* and breed as such. Even though they carry *C*, a gene for pigment, they lack the extension factor *E* and the pattern factor*L* upon which black pigment is superimposed.

APPLICATION OF THEORY TO BREEDING OPERATIONS

Theoretically the most desirable kind of "standard" color Rhode Island Red is the one in which genes *B* and *L* are combined. The genotypes would then be *BBLLee*, *BBLlee* males and *BBLoee* females. In such birds there should be no smut and there should be a rich red color throughout the plumage. Gene *B* behaves as a cumulative gene so that birds must be homozygous for *B* in order to be deep red. Birds heterozygous for *B* are light in shade of red. Individuals carrying genes *B* and *E* are less desirable for two reasons; first, there will be stippling in plumage; second, smutty progeny result whenever factor *E* is brought in contact with factor *L* or with both *B* and *L*. Females of the composition *BBLoee* would not breed true for Red because half their female progeny would be white when mated to *BBLl* males. Such females could be recognized if mated to males of known genetic composition. *BBLoee* females, if mated to a "standard" smutty male *BBLlEe*, would give 25% white female progeny. If the male were homozygous for *E* (*BBLlEE*) there would be no white progeny. As the breed has progressed toward darker color, it is probable that most females as well as males carry gene *E*. This will account for the absence of white progeny and for the frequent occurrence of smut in any "standard" flock.

The crucial test for the selection of the breeding male is largely his phenotype or actual color. If he is rich bay in color throughout his feathers and is absolutely free from smut or stippling in any part, he should be either a *BBLLee* or a *BBLlee* bird. If in the breeding test using only females with deep bay surface color gives some white progeny, the probabilities are that the male is heterozygous for *L*. If the breeding test gives some smutty or stippled progeny, the gene *E* must be present in the female breeders.

The crucial test in the selection of female breeders is the individual color pattern. Deep bay, non-stippled, non-smutty females with red under-color are of only one genotype, *BBLoee*. Such females will not give smutty or stippled progeny unless gene *E* is introduced by their mate. Females may be deep bay in surface plumage but stippled with brown or black because of gene *E* or they may be smutty below the surface because they carry genes *B*, *L*, and *E*. The progeny of these *E*-bearing females will exhibit either stippling in females or smut in both males and females.

In breeding for rich bay plumage color with the columbian pattern, (gene e^m restricting black pigment to wings, tail and hackle) it becomes apparent that what is most necessary is the intensifying of brown color by the proper combination of genes *B* and *L*. The latter gene in addition to affecting color distribution, seems to intensify the shade of red so that *BBLL* birds are very dark while *BBllEe* birds are only medium red. *BbLLee* birds are too light in shade of red since they carry gene *B* in heterozygous condition. Another problem is to eliminate the gene *E* so that stippling or mottling and smutty under-color may no longer occur.

CONCLUSIONS

1. Rhode Island Red color pattern is a complex of several factors. Three factors are chiefly concerned in the make-up of the different phenotypes.

2. Bay pigment depends on the presence of a gene *B*, which is autosomal and epistatic to gold (*s*).

3. The sex-linked pattern factor *L* carries gold and to some extent influences the distribution of pigment and its intensity.

4. The autosomal gene *E* may or may not be present and determines the presence of smutty color in feather fluff.

5. Gene *E* is not essential in breeding for "standard" color because genes *B* and *L* together give the ideal color pattern.

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THE EFFECT OF ENVIRONMENT ON VARIEGATION PATTERNS IN MAIZE PERICARP[1]

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STATEMENT OF THE PROBLEM

EMERSON (1914, 1917) was the first to call attention to the remarkable series of multiple allelomorphs concerned with the development and distribution of color in maize pericarp. These allelomorphs include genes for self red, self orange, white-capped red, basal red, and colorless pericarp, associated with either self red or white cobs, and at least three types of variegated pericarp associated with similarly variegated cobs. The principal difference between these genes was thought to lie in their relative frequencies of mutation. It was suggested that the several types of variegated pericarp differ in frequency of factorial change by virtue of the fact that the change begins earlier in some types than in others.

HAYES (1917) made a study of a coarse type of variegation in maize, known as mosaic pericarp, with results quite similar to those found by EMERSON in the calico type of variegation. HAYES intercrossed the various allelomorphic types and concluded that certain combinations produce germinal instability.

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[1] This investigation was made in the Department of Field Crops, MISSOURI AGRICULTURAL EXPERIMENT STATION. The experiments were conducted at Sacaton, Arizona, under the supervision of C. J. KING, and at Chula Vista, California, under the supervision of C. G. MARSHALL, through the courtesy of the Office of Crop Acclimatization of the BUREAU of PLANT INDUSTRY.

EYSTER (1924) was able to demonstrate the suggestion made by EMERSON that the large series of multiple allelomorphs is evidence that a fundamental gene concerned in the development of pericarp and cob colors and color pattern has at one time or another mutated in diverse ways. A single self orange ear, in the course of a few generations in pedigreed cultures, gave rise to a large series of multiple allelomorphs, including strains of self orange varying in color intensity from whitish to deep red, self red, white-capped red, colorless and a relatively large number of distinct types of variegation, ranging in color pattern from ears that are almost colorless to ears that approach the self red ears in appearance.

It has also been found (EYSTER 1925) that the fundamental gene for mosaic pericarp changes in such a way as to give rise to pericarp and cob colors and color patterns which are in every respect parallel to those which occur in the calico type of variegation.

The detailed studies of both the calico and the mosaic series of multiple allelomorphs led the writer (loc. cit.) to the following general conclusions: (1) Self orange color is the expression of a gene which includes in its structure gene elements for red pericarp associated with gene elements for colorless pericarp, and (2) a variegation is produced when the contrasting gene elements become segregated by the mechanism of mitosis in the course of pericarp development.

The idea that a variegation is the result of the mitotic segregation of elements within the gene suggested the question as to the possible effects of different environmental conditions on the process. Accordingly, as a preliminary experiment, plantings from each of a number of variegated and self orange ears were made in localities which differ strikingly in such environmental conditions as might be expected to affect growth and development, and the present paper is a report of the results that have been found.

MATERIALS AND METHODS

The materials used in this experiment were prepared by crossing the plants having the pericarp color or pattern to be studied with a strain having colorless pericarp but red cob, and then back-crossing such F_1 plants with a strain having colorless pericarp and colorless cob, according to the method described elsewhere (EYSTER, 1924). The kernels of the variegated ears prepared in this manner were of two sorts as regards their genetic constitution, as follows: $\frac{\text{W R}}{\text{W W}}$ and $\frac{\text{V V}}{\text{W W}}$. Accordingly, approximately one-half of the plants produced ears with colorless pericarp and red cob, while the remainder of the plants produced ears with variegated pericarp and cob or some other allelomorphic type due to a genetic change in the *V V* gene. Likewise the kernels of the orange ears were of two sorts as regards their genetic constitution as follows: $\frac{\mathrm{W\ R}}{\mathrm{W\ W}}$ and $\frac{\mathrm{O\ O}}{\mathrm{W\ W}}$. In this case also, approximately one-half of the plants produced ears with colorless pericarp and red cob, while the remainder of the plants produced ears with orange pericarp and cob or an allelomorphic type due to a genetic change in the *O O* gene. By this method it was possible to identify the gene under study, regardless of what allelomorphic changes it may have undergone. In each case the ears with colorless pericarp and red cob, of the constitution $\frac{\mathrm{W\ R}}{\mathrm{W\ W}}$, were discarded.

It should be kept in mind that a single gene is responsible for the development and distribution of pigment in both pericarp and cob. The genes which have been referred to above are the following:

	Expression in	
Symbol	Pericarp	Cob
W R	Colorless	Red
W W	Colorless	Colorless
V V	Variegated	Variegated
O O	Orange	Orange

Eight variegated and two orange ears were used in the experiment. The kernels of each ear were removed from the cob separately and divided into three equal lots, one lot to be planted in each of the localities chosen for growing the plants.

LOCALITIES IN WHICH THE PLANTS WERE GROWN

One lot of the kernels from each ear was grown in each of the following localities: Columbia, Missouri; Chula Vista, California; and Sacaton, Arizona.

Columbia, Missouri was chosen, largely as a matter of convenience, as a location where the environmental factors might be considered best suited for the normal growth and development of maize. Due to unfavorable cultural conditions and unusual chinch-bug injury, very few ears were matured in this locality, though more than 2500 plants were grown. Such ears as were produced in each of the progenies were quite like those grown at Chula Vista, California.

The planting at Chula Vista, California was made early in June, and the matured ears were harvested OCTOBER 15, making a growing season of approximately four months. During this period the air temperature seldom exceeded 75 degrees *F* and the mean daily fluctuation was not much over 20 degrees *F*.[2] The soil temperature had a daily range of from 60 degrees *F* to 80 degrees *F* and was very uniform. The radiation is less than that of the corn belt region due to the high relative humidity.

The planting at Sacaton, Arizona was made July 11 and the ears were fully matured October 15, making a growing season of a little more than three months. The air temperature was quite variable, ranging from 60 degrees *F* at night to as much as 110 degrees *F* during the day. The soil temperature was more variable, changing with the irrigations. The atmosphere at Sacaton is typical southwestern desert air, with a low relative humidity and a high radiation.

The soils in which the plantings were made at Chula Vista and Sacaton are quite comparable, both being alkaline and somewhat impervious. Both crops were grown under irrigation. The water used in irrigation at Chula Vista was surface water stored during the winter from mountain streams, while the water used at Sacaton was taken from wells and was distinctly salty. Both plots were irrigated before water stress conditions developed so that soil moisture conditions can be considered as having been optimum.

The chief environmental difference between Chula Vista and Sacaton seems to have been the higher and more variable temperature and greater transpiration at Sacaton. As a consequence of this difference the season required to mature the pericarp at Sacaton was fully a month shorter than that required at Chula Vista.

THE EFFECT OF ENVIRONMENTAL DIFFERENCE ON THE VARIEGATION PATTERN

The variegated ears grown at Chula Vista, in a cool, moist climate, where the growing season was relatively long, developed a fairly coarse pattern of variegation in the form of splashes, bands, and larger segments of the surface of a kernel, while the remainder of the pericarp appeared perfectly colorless. Variegated ears of the same genetic constitution and taken from the same parent ear but grown at Sacaton, in a region with a hot, dry climate and a short growing season, developed a strikingly different variegation pattern. The variegation consists in a smaller number of red areas which cover one-fourth and larger parts of the surface of a kernel, but a larger number of smaller red areas in the form of splashes, lines, and bands. The general surface of the kernels, not occupied by the red markings of the variegation is not colorless in the Arizona-grown ears as in the California-grown ears, but has a more or less distinct washed-out-

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[2] The writer is indebted to J. H. Kempton of the Office of Crop Acclimatization of the Bureau of Plant Industry for the detailed statements concerning the environmental conditions existing at Chula Vista, California, and at Sacaton, Arizona.

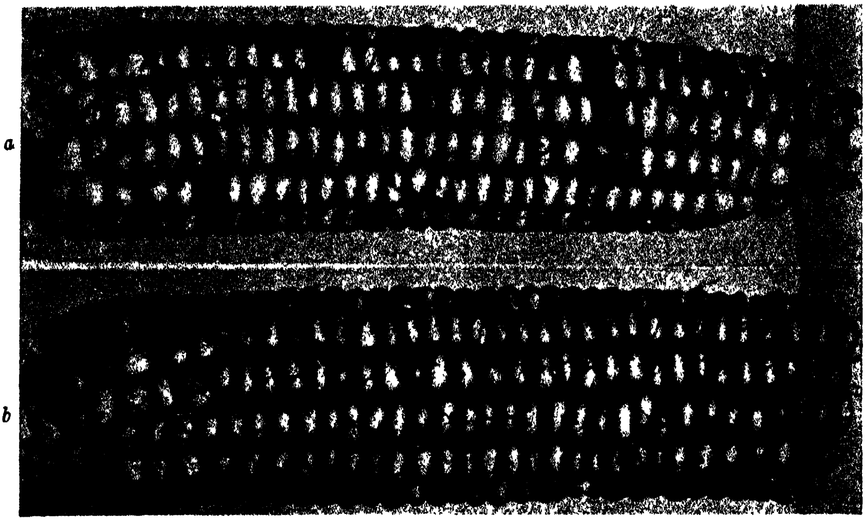

FIGURE 1.—Typical ears showing the variegation patterns of ears of the same strain grown under different environmental conditions. (*a*) Pattern which developed under Chula Vista conditions. (*b*) Pattern which developed under Sacaton conditions.

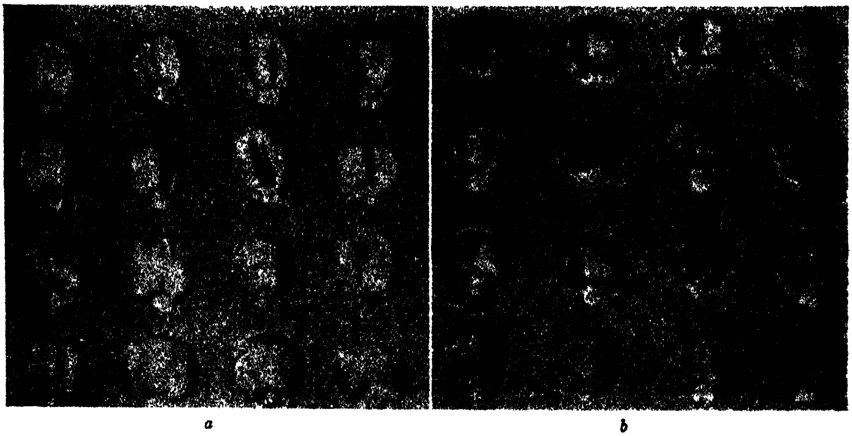

FIGURE 2.—(*a*) Representative kernels from ear shown in Fig. 1 a. (*b*) Representative kernels from ear shown in Fig. 1 b.

red color. Accordingly, all of the Arizona-grown variegations consist of relatively small areas of red on a washed-out-red background. The difference in the variegation pattern under the conditions of these two environments was consistent in all of the plantings that were made, as may be seen from the accompanying illustrations.

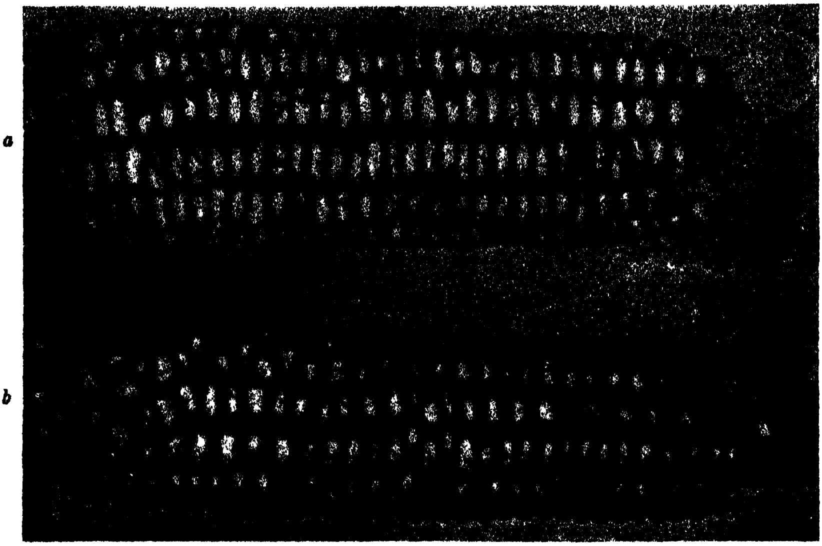

FIGURE 3.—Typical ears showing the variegation patterns of ears of a second strain grown under different environmental conditions. (*a*) Pattern which developed under Chula Vista conditions. (*b*) Pattern which developed under Sacaton conditions.

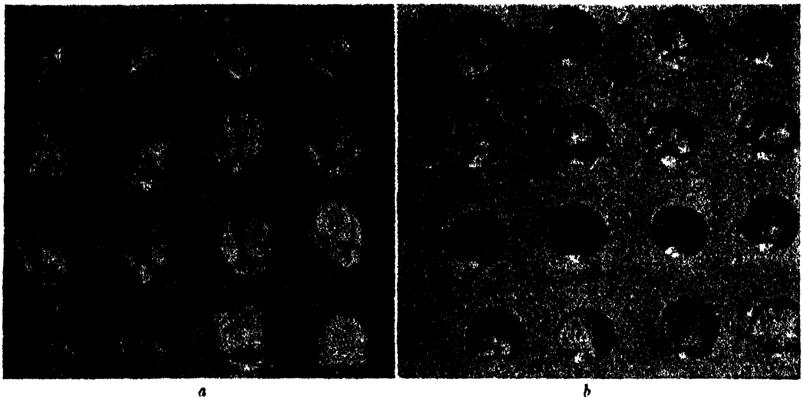

FIGURE 4.—(*a*) Representative kernels from ear shown in Fig. 3 *a*. (*b*) Representative kernels from ear shown in Fig. 3 *b*.

Kernels taken from the same parent ear were grown at Sacaton under number 2986 and at Chula Vista under number 2997. The ear shown in figure 1*a* illustrates the variegation pattern typical of the ears grown at Chula Vista, and ear figure 1*b* is representative of the ears grown at Sacaton. A comparison of typical kernels taken from these ears is shown in figure 2. The kernels grown at Chula Vista are shown in figure 2*a*, and those grown at Sacaton in figure 2*b*. The pattern differences which have already been described are apparent in these illustrations.

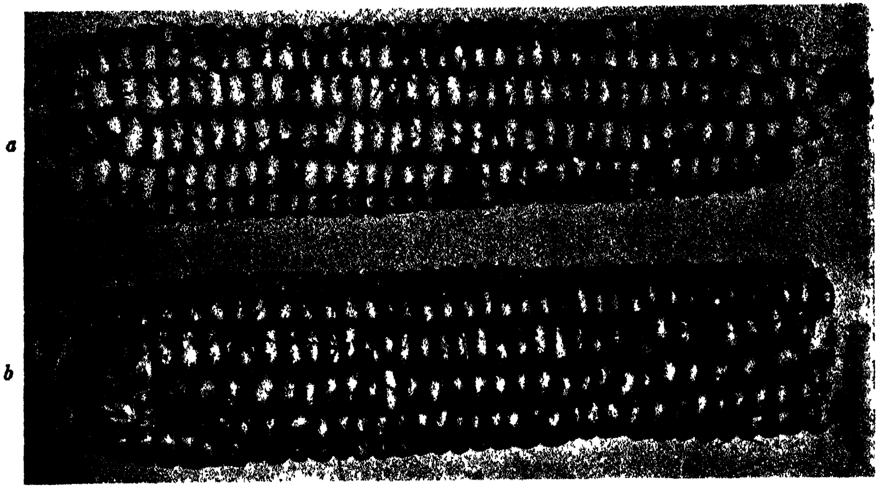

FIGURE 5.—Typical ears showing the variegation patterns of ears of a third strain grown under different environmental conditions. (*a*) Pattern which developed under Chula Vista conditions. (*b*) Pattern which developed under Sacaton conditions.

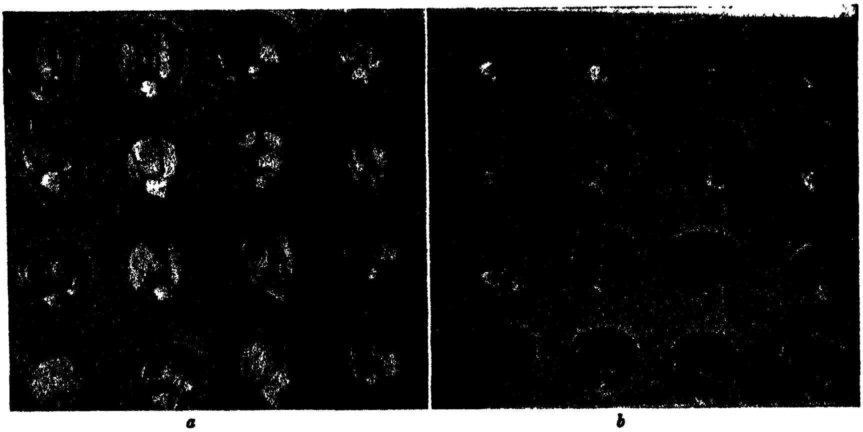

FIGURE 6.—(*a*) Representative kernels from ear shown in Fig. 5 *a*. (*b*) Representative kernels from ear shown in Fig. 5 *b*.

Kernels taken from a second variegated ear were grown at Chula Vista under number 2998 and at Sacaton under number 2987, and a typical ear from each planting is shown in figure 3. Typical kernels from these ears are illustrated in figure 4. The relatively large areas of red on a colorless background are characteristic features of the California-grown ears, and are in striking contrast with the fine markings and residual color in the pericarp of the Arizona-grown ears.

From a third variegated ear a planting was made at Chula Vista under number 2992 and at Sacaton under number 2982, and a representative ear from each planting is shown in figure 5. Representative kernels from each of these ears are illustrated in figure 6. Again the variegation patterns are strikingly different. Similar differences were found also in each of the other plantings that were made in connection with this experiment.

FREQUENCY OF OCCURRENCE OF SELF RED AREAS IN VARIEGATIONS UNDER THE CONDITIONS OF THE DIFFERENT LOCALITIES

The variegated ears were first studied for areas of red extending over more than a single kernel. The kernels were then shelled off the ear and studied individually and classified according to the size of the largest red area on each kernel.

California-grown Ears

From five to ten percent of the variegated ears grown in this locality had an exceedingly light pattern, while the remainder of the ears were like those shown in the illustrations above. The ears were separated into those which had the very light pattern and those which had the medium pattern like the parent ears before the data on the number and size of the red areas were taken.

A summary of the red areas found on the ears with the extremely light variegation pattern is given in table 1.

TABLE 1

PEDIGREE	VARIEGATED KERNELS	CHANGES TO RED IN KERNELS				
		¼	½	¾	1	>1
2997	2163	3	2	0	0	0
2998	1710	2	5	1	0	0
2990	2604	15	6	1	0	0
2992	203	7	3	1	1	0
2993	1126	3	4	2	1	0
2995	1178	3	2	0	0	0
Total	9584	33	22	5	2	0
percent	99.36	0.34	0.23	0.05	0.02	0.00

Two red areas were as large as a single kernel, but none were found that were larger than this. Less than one percent of the kernels had red areas that were as large as one-fourth of the surface of a single kernel.

The data on the number and size of the red areas that occurred on the more heavily variegated California-grown ears are given in table 2.

TABLE 2

PEDIGREE	VARIEGATED KERNELS	CHANGES TO RED IN KERNELS				
		¼	½	¾	1	>1
2997	8654	150	40	25	13	1
2998	6848	118	40	24	6	4
2990	10418	270	62	20	3	2
2992	5898	227	64	40	21	3
2993	4491	189	60	45	20	5
2995	6555	216	105	48	23	4
Total	42864	1170	371	202	86	19
percent	95.87	2.62	0.83	0.45	0.20	0.04

Nineteen red areas extended over more than a single kernel, and 4.13 percent of all the kernels had red areas that covered one-fourth the surface of a single kernel or larger areas.

Arizona-grown Ears

The variegated ears grown in Arizona did not include a single ear with the extremely light pattern found among the California-grown ears. The patterns were all fundamentally like those shown in the illustrations which have already been described, with variations in the number and size of the red areas and in the amount of residual color in the general pericarp.

A summary of the red areas that occurred on the variegated ears grown in Arizona is given in table 3.

Comparison of the Occurrence of Red Areas in California-and Arizona-grown Variegations

It should be kept in mind that the California-grown variegations were of two distinct patterns: (1) a medium heavy pattern, similar to that of the parent ear, and (2) an extremely light pattern. The variegations grown in Arizona were of one general type and consisted of many relatively fine red markings on a reddish background. A comparison in the frequency of the occurrence of red markings which covered one-fourth of a kernel and larger areas in the heavy variegations grown in the two localities is shown in table 4.

In every planting that was made and studied there were more self red areas covering one-fourth of a kernel or larger areas in the California-grown variegations than in the corresponding variegations grown in Arizona. The differences range from 0.07 ± 0.17 to 2.56 ±0.27. In four of the six plantings, the differences are from seven to a little more than ten times their probable errors and are doubtless significant. A total comparison of the six plantings shows that the percent of self red areas covering one-fourth or more of the surface of a kernel was 4.14 ± 0.07 under the California conditions and 2.86 ± 0.05 under the Arizona conditions. This is a difference of 1.28 ± 0.086. This difference is approximately 14.5 times as large as its probable error.

TABLE 3

PEDIGREE	VARIEGATED KERNELS	CHANGES TO RED IN KERNELS				
		¼	½	¾	1	>1
2986	9254	96	21	16	18	1
2987	9624	154	51	39	19	0
2980	12211	143	60	22	5	1
2982	6215	107	58	19	16	0
2983	7123	246	105	41	37	4
2985	4500	92	43	16	13	0
Total	48927	838	338	153	108	6
Percent	97.14	1.66	0.67	0.30	0.22	0.01

TABLE 4

PEDIGREE.		CHANGES TO RED COVERING ¼ KERNEL AND LARGER AREAS, IN PERCENT.		DIFFERENCE
California	Arizona	California	Arizona	
2997	2986	2.58± .11	1.62± .09	0.96± .14
2998	2987	2.73± .13	2.66± .11	0.07± .17
2990	2980	3.31± .12	1.86± .08	1.45± .14
2992	2982	5.68± .20	3.12± .18	2.56± .27
2993	2983	6.63± .24	5.73± .18	0.90± .30
2995	2985	5.73± .19	3.52± .18	2.21± .26
Average		4.14±0.07	2.86±0.05	1.28± .086

The objection might be made that by excluding the very light-patterned ears of the California-grown material an unfair comparison is made. Consequently in table 5 is given a summary of all the California-grown variegations, including the extremely light variegated ears, in comparison with the Arizona-grown variegations.

TABLE 5

PEDIGREE		CHANGES TO RED COVERING ¼ KERNEL AND LARGER AREAS, IN PERCENT.		DIFFERENCE
California	Arizona	California	Arizona	
2997	2986	2.12±0.09	1.62±0.09	0.50±0.13
2998	2987	2.30±0.11	2.66±0.11	−0.36±0.15
2990	2980	2.83±0.09	1.86±0.08	0.97±0.12
2992	2982	5.67±0.19	3.12±0.18	2.55±0.26
2993	2983	5.53±0.20	5.73±0.18	−0.20±0.27
2995	2985	4.59±0.15	3.52±0.18	1.07±0.23
Average		3.51±0.05	2.86±0.05	0.65±0.07

Again in four of the six plantings the California-grown kernels had a larger percent of kernels with red areas that covered one-fourth or more of the surface of a kernel. The differences in percent range from four to nearly ten times their probable errors, even when the extremely light variegated ears of the California-grown plants are included. The differences in families 2998–2987 and 2993–2983 are in favor of the Arizona-grown ears but are not significant.

When the extremely light and heavy variegations of the California-grown kernels are considered together, the self red areas amount to 3.51 ± 0.05 percent, as compared with 2.86 ± 0.05 percent in the Arizona-grown variegations. This is a difference of 0.65 ± 0.07 . This difference is a little more than nine times its probable error.

The result of this comparison is that the California-grown kernels have more red markings that cover one-fourth the surface of a kernel and larger areas than the Arizona-grown variegations.

VARIEGATED AREAS ON SELF ORANGE KERNELS

Orange kernels, of the genetic constitution that has already been indicated, were taken from the same ear and divided into three lots, one of which was planted in Missouri under number 2974, the second lot was planted in Arizona under number 2984, and the third lot was planted in California under number 2994. The number of ears that were produced from the planting in Missouri was too small for further study. The ears of the Arizona and California plantings varied from whitish to deep orange or red, and the orange pericarp changed to variegation and self red in the manner described previously (Eyster, 1925). No color changes were larger than the surface of a single kernel.

The kernels were removed from the ears and classified according to t presence and size of variegated areas as given in table 6.

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Table 6

	Changes to variegation.			Total
	Orange kernels	Band to ¼ kernel	¼ to entire kernel	
California grown	14462	1492	153	16107
Arizona grown	14412	1252	90	15754

The relative frequencies of the changes from self orange to variegated pericarp in the California-and Arizona-grown ears may be seen more clearly from the comparison expressed in percent of the total number of kernels given in table 7.

Table 7

	Percent of total kernels			Total
	Orange kernels	Changes to Variegation		
		Band to ¼ kernel	¼ to 1 kernel	
California grown	89.79	9.26±0.16	0.95±0.05	10.21±0.16
Arizona grown	91.48	7.95±0.15	0.57±0.04	8.52±0.16
Difference	−1.69	1.31±0.22	0.38±0.06	1.69±0.23

The variegations which covered from a band to one-fourth of the surface of a kernel occurred 1.31 ± 0.22 percent more frequently in the pericarp of the California-grown ears. This difference is about six times its probable error. The variegations that extended over as much as one-fourth the surface of a kernel and larger areas could be recorded accurately, and it was found that the California-grown ears had 0.38 ± 0.06 percent more of these larger areas than the Arizona-grown ears. This difference is a little more than six times its probable error.

This comparison indicates that changes in pericarp color from orange to variegation occurred more frequently under the California conditions.

RED AREAS ON SELF ORANGE KERNELS

A record was also made of the color changes from orange to self red which covered a longitudinal band of the surface of a kernel and larger areas. No red area was found that extended over more than a single kernel. summary of the red areas which occurred on orange pericarp is given in le 8.

TABLE 8

	CHANGES TO SELF RED			TOTAL
	Orange kernels	Band to ¼ kernel	¼ to entire kernel	
California grown	15059	934	114	16107
Arizona grown	15361	342	51	15754

A comparison of the frequency of occurrence of red areas in orange pericarp between the California-grown and Arizona-grown ears, expressed in percent of the total number of kernels observed, is given in table 9.

TABLE 9

	PERCENT OF TOTAL KERNELS			TOTAL
	Orange kernels	Changes to Self Red.		
		Band to ¼ kernel	¼ to 1 kernel	
California grown	93.56	5.80±0.12	0.71±0.04	6.51±0.13
Arizona grown	97.50	2.18±0 08	0.32±0.03	2.50±0.09
Difference	−3.94	3.62±0.14	0.39±0.05	4.01±0.16

The red areas which covered from a longitudinal band to one-fourth of the surface of a kernel occurred 3.62=0.14 percent more frequently in the pericarp of the California-grown ears. This difference is a little more than twenty-five times its probable error. The red areas which extended over areas larger than one-fourth of the surface of a single kernel occurred 0.39=0.05 percent more frequently under the California conditions. This difference is approximately eight times its probable error.

From these data it is to be concluded that the changes in pericarp color from orange to red occurred more frequently under the California conditions as compared with the conditions in Arizona.

DISCUSSION

In previous studies on variegation, especially those mentioned in an early part of this paper, results were reported which suggest that a variegation is caused by the segregation of contrasting substances in the same gene. The size of a color change is large or small depending upon whether the necessary segregation within the variegation gene occurs earlier or later in development. As was pointed out by EMERSON, the probability of pericarp color changes effecting the germ-plasm and thus becoming genetic increases as they extend over larger segments of the surface of a kernel. The higher percents of color changes extending over one-fourth of the surface of a kernel and larger areas in the variegated and self-orange strains grown in California, as compared with the same strains grown in Arizona, indicate that genetic changes occurred more frequently under the California conditions. Since the genetic constitution of the several strains grown in the two localities were identical, it must be concluded that the observed differences in the frequency of genetic changes were due to the differences in the environmental conditions of the two localities.

Although the Arizona-grown variegations have fewer red markings that extend over relatively large areas, they appear to have more total color than the California-grown variegations. This suggests that the minimum amount of the pigment-producing component of the variegation gene necessary to cause pigment to develop in the pericarp may vary under different environmental conditions. According to this view, some of the segments which become pigmented in Arizona remain colorless in California.

These preliminary studies indicate rather definitely that environmental factors not only may modify the expression of a character in the soma, but may also have a direct influence on the germ-plasm.

SUMMARY

1. Strains of maize with variegated pericarp and strains with orange pericarp were grown under strikingly different environmental conditions as a preliminary study of the relation of environmental factors on the somatic expression and genetic changes of these pericarp color patterns.
2. Each strain was grown at Chula Vista, California and at Sacaton, Arizona. The chief environmental difference between these two localities seems to have been the higher and more variable temperature and greater transpiration at Sacaton. As a consequence of this difference the season required to mature the pericarp at Sacaton was fully a month shorter than that required at Chula Vista.

3. Eight variegated strains were grown at each of these localities, and in every case there was a definite and consistent difference in the variegation pattern.

4. The California-grown variegations consistently had more red markings covering one-fourth the surface of a kernel and larger areas than the Arizona-grown variegations. The significance of this difference is that under the California conditions more of the color changes extended into the germ-plasm and thus became genetic.

5. Changes from orange to variegation, and from orange to red also occurred more frequently under the California conditions.

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LINKAGE STUDIES WITH THE TOMATO

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INTRODUCTION

Experimental genetic data have been collected systematically for several years from the writer's crosses of varieties of the garden tomato, *Lycopersicum esculentum*. These studies have involved many of the most common differential characters of the species, both qualitative and quantitative, commercial and otherwise. Of the more qualitative characters nine are sufficiently analysed to warrant a report of findings as to the manner of their inheritance. Other qualitative characters, some new mutants, and a number of more difficult size and shape complexes are being subjected to further analysis.

Genetics of the tomato, besides being of ultimate commercial importance, has an immediate and special scientific interest, since the species has many closely related species, an abundance of varieties, an exceptionally wide range of size and shape differences in fruits suitable for quantitative studies, rather frequent mutations both by seed and by bud, and a reasonably small chromosome number ($n=12$), and is frequently used in studies of selective fertilization, graft-hybridization (WINKLER, 1916), and triploidy and tetraploidy (most recently by LESLEY and MANN

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1925, and Mrs. LESLEY 1925). It thus promises contributions toward the solution of many current genetic problems from nearly all angles of approach.

The present report is designed to help establish the fundamental genetic pattern of the species. Accordingly, first attention has been given to the question of the independence or association in inheritance of certain well-defined factors.

DESCRIPTION OF CHARACTERS AND VARIETIES USED

The manner of inheritance of many characters of the tomato has been determined in part, and often only in a preliminary way, by earlier investigators chiefly from the AGRICULTURAL EXPERIMENT STATIONS, that is (HALSTED 1905, 1918), HURST (1906), CRAIG (1907), HEDRICK and BOOTH (1907), PRICE and DRINKARD (1908), GILBERT (1912), GROTH (1910–1915), CRANE (1915), FRIMMEL (1922), LINDSTROM(1924–1925), and WARREN (1925). The meagre and more or less unsatisfactory data were summarized for indications of linkage up to 1917 by JONES and up to 1925 in LINDSTROM's more critical paper.

The characters I am reporting here concern differences in stature, fruit colors, fruit skin surface, distinct fruit shapes, form of inflorescence, foliage color and shape of leaf. In the brief descriptions which follow of the 9 distinct contrasting allelomorphic pairs, the abbreviations designating factors are, whenever possible, in order to prevent unnecessary future confusion, taken from former published work.

1. Stature (Vine habit). Tall (D) dominant over dwarf (d). Dwarf plants differ from the tall in having an upright habit of growth, stocky shortened stems, short broad cotyledons, leaflets and petioles, peculiar rugose, puckered and down-curved leaves, and a deeper green foliage color.

2. Fruit Skin Structure. Smooth (P) is dominant over "peach" (p). The "peach" fruit epidermis possesses a persistent pubescence and a glandular structure making the fruit tomentose and dull as compared with the smooth and shining appearance of fruit of other varieties. The gene p also produces some extra pubescence and a blue-gray cast over the entire plant.

3. Fruit Shape. Mature fruit without constricted neck (P_r) is dominant over pear-shaped mature fruit (p_r). Some confusion has formerly resulted from attempts to classify plants for these characters before their fruits attain maturity. Fruits of oval or plum shape often pass through an unripe stage resembling the true pyriform. Certain evidence suggests also that related fruit forms, such as "plum" and the slender-necked "calabash", carry factors allelomorphic with P_r and p_r.

4. Inflorescence Type. Simple inflorescence, arising from successive third internodes (*S*) is dominant over the compound, repeatedly branching, more or less leafy type arising from successive sixth nodes (*s*). Such a compound form with 30 or more flowers and fruits per cluster is found in one American variety, Grape Cluster, and in a few European varieties (CRANE 1915).

5. Fruit Flesh Color. Red flesh (*R*) is dominant over yellow (*r*). In mature fruit cells the gene *R* controls the formation of a red pigment (*lycopersicin*) in needle-like crystals, while its absence, or the presence of *r*, results in yellow granules. In "albino" fruited varieties the flesh color may possibly be a slightly paler yellow.

6. Fruit Skin Color. Presence of a yellow-orange pigment in epidermal cell walls of ripe fruit (*Y*) is dominant over the clear, colorless state (*y*).

7. Foliage Color. The ordinary fully-green foliage (*G*) is dominant over the pale yellow-green condition (*g*) met with in Honor Bright, whose new growth, at first green, soon becomes yellowish from below upward. Immature fruits of yellow-green plants remain characteristically pale white until just before ripening to their definitive color.

8. Leaf Shape. The deeply dentate leaflet of common "cut leaf" varieties (*C*) is partially dominant over "potato-leaf" (*c*) whose leaflets have nearly entire margins.

9. Fruit Shape. Fruits with smooth regular outline (*F*) are dominant over those of Albino, a Ponderosa type, with distinctly lobulated irregular contour (*f*), the irregularity even extending to the extreme of producing compound fruits by fusion of as many as four. This roughness of outline is anticipated even in the flower by a strongly correlated flattening of the pistil, a high number of petals and sepals (8–10 or more instead of 5–7) and in the fruit by a very large number of ovarian locules. The genes *Ff* are probably one of the complementary pairs described by WARREN (1925) as controlling fasciation, but in the absence of published quantitative data in his paper it is not yet possible to homologize the genes definitely.

The ten varieties (Vaughan's, Ferry's and Steele-Briggs') used containing these characters were all bagged and selfed for 2–5 years before crossing, and all proved homozygous for the characters dealt with. The genetic formulae assigned to these varieties below describe them most briefly:

Variety and Abbreviation		Constitution								
Dwarf Aristocrat	(Dw Ar)	DD	PP	P_rP_r	SS	RR	YY	GG	CC	FF
Pink Peach	(P Pch)	DD	$\underline{pp}$	P_rP_r	SS	RR	$\underline{yy}$	GG	CC	
Yellow Pear	(Y Pr)	DD	PP	p_rp_r	SS	rr	YY	GG	CC	FF

Variety and Abbreviation		Constitution								
McMullens Pink	(McM P)	*DD*	*PP*	P_rP_r	*SS*	*RR*	*yy*	*GG*	*cc*	*FF*
Albino	(Alb)	*DD*	*PP*	P_rP_r	*SS*	*rr*	*yy*	*GG*	*CC*	*ff*
White Apple	(Wh App)	*DD*	*PP*	P_rP_r	*SS*	*rr*	*yy*	*GG*	*CC*	*FF*
Honor Bright	(H Br)	*DD*	*PP*	P_rP_r	*SS*	*RR*	*YY*	*gg*	*CC*	*FF*
Golden Queen	(G Q)	*DD*	*PP*	P_rP_r	*SS*	*rr*	*yy*	*GG*	*CC*	*FF*
Red Currant	(R C)	*DD*	*PP*	P_rP_r	*SS*	*RR*	*YY*	*GG*	*CC*	*FF*
Grape Cluster	(G Cl)	*DD*	*PP*	P_rP_r	*ss*	*RR*	*YY*	*GG*	*CC*	*FF*

Since any one commercial variety seldom contains many recessive factors it was necessary to make scores of crosses to obtain all the desired combinations. From F_2 recombinations and successive synthetic crosses many new poly-recessive types are now available, including linkage testers for back-crossing.

The Unifactorial Nature of these Characters

That each of the characters described above is controlled by a single factor pair is quite fairly demonstrated from much F_2 and back-cross data in the crosses involving these allelomorphs. Experience has shown that dominance is nearly complete in the first generations of all, that the parental phenotypes reappear well-defined in the F_2 generation giving a clear segregation on a monohybrid basis, and that extracted recessives breed true in progeny tests.

The segregation is apparent from observation alone, but in all cases where fruit shapes and sizes are concerned confirmatory check measurements of polar and equatorial diameters and average fruit weights of all plants in all generations were also taken. Such data will be reported in later quantitative studies.

It might be noted that the data summarized in table 1 are the same as those used in dihybrid form for linkage tests (page 391) and include every classified individual of the F_2 generations bred, there being practically no mortality after germination.

TABLE 1

Monohybrid ratios obtained in F_2 populations

CHARACTERS AND FACTORS		F_2 PHENOTYPES		DEVIATIONS FROM EXPECTED 3:1 RATIOS
		D	*R*	
Tall (*DD*)	× dwarf (*dd*)	937	336	17.75±10.6
Smooth (*PP*)	× peach (*pp*)	976	324	1.0 ±10.51
Not pear (P_rP_r)	× pear (p_rp_r)	959	326	4.75±10.5
Simple (*SS*)	× compound (*ss*)	84	24	4.0 ± 2.93
Yellow (*YY*)	× clear (*yy*)	2339	725	41.5 ±15.86
Red (*RR*)	× yellow (*rr*)	1737	590	8.2 ±14.15
Cut leaf (*CC*)	× potato (*cc*)	560	172	11.0 ± 7.82
Green (*GG*)	× yellow (*gg*)	873	286	3.75± 9.89
Regular (*FF*)	× fasciated (*ff*)	305	111	7.0 ± 6.08

Since the deviations from the theoretical 3:1 ratios seldom much exceed those to be expected from random sampling, there can be no reasonable doubt that we are dealing in all these cases with single factor differences. The rather large departure from expectation in the crosses of yellow-skinned (*YY*) with clear-skinned (*yy*) varieties may signify a slight inviability of the recessive type or a small amount of selective fertilization favoring the *Y* gene.

TESTS FOR LINKAGE OR INDEPENDENCE OF GENES

The dihybrid and polyhybrid crosses so far carried through the F_2 generation furnish linkage tests covering 24 different combinations of these genes taken two at a time. The F_2 classifications were made by the writer and independently checked by Mr. CECIL DUFF, who gave valuable assistance in the work for three successive summers.

Four of the combinations of pairs of genes gave clear indications of linkage, while the remaining twenty combinations afford equally clear evidence of free assortment. The cases showing linkage will be first described.

THE FIRST LINKAGE GROUP

The first linkage group in the tomato contains genes *Dd*, *Pp*, P_rp_r, and *Ss*. In the four crosses made involving two pairs of these genes at a time, in each instance one pair of genes entered with one parent and the other with the second; accordingly, the resulting F_2 ratios fit into the "repulsion" series. The linkages are close enough to be indisputable even without more critical backcross data, for the percentage of crossing over in no case exceeds 20 percent.

1. Dwarf Aristocrat (*dd PP*) times Pink Peach (*DD pp*). From this cross and its reciprocal all F_1 plants (*Dd Pp*) were tall and bore smooth fruits. The F_2 generation produced only three of the expected four classes (table 2).

TABLE 2

Data showing repulsion between d and p genes

CROSSES	F_2 CLASSES (PHENOTYPES)			
	DP	*Dp*	*dP*	*dp*
dd PP × *DD pp*	135	52	53	0
DD pp × *dd PP*	116	58	54	0
Total	251	110	107	0

This ratio evidently corresponds to a 2:1:1:0 ratio, suggesting very close or complete linkage (repulsion) between genes *Dd* and *Pp*. Presumably owing to this fact there is no known "dwarf peach" commercial variety of the tomato. Whether the linkage is actually complete, larger F_2 generations should determine. To date only 677 F_2 individuals from this cross have been reported classified, 468 in the present case and 209 in a paper by Lindstrom (1925). The latter were distributed in a similar ratio: 101 *DP*:56 *Dp*:52 *dP*:0 *dp*, again indicating complete linkage; but from one of his F_2 plants (open pollinated) there segregated two double recessive, dwarf peach plants!

2. Dwarf Aristocrat (ddP_rP_r) × Yellow Pear (DDp_rp_r). The F_1 plants (DdP_rp_r) were tall in stature and produced small, more or less elongated fruits without constricted necks. Their progeny fell into four distinct classes as regards these characters: 106 tall, not pear (DP_r). 58 tall, pear (Dp_r) 57 dwarf, not pear (dP_r), and 1 dwarf pear (dp_r). From the reciprocal cross it is known only that 2 dwarf pear plants appeared in a total of 250 F_2 individuals.

The ratio, 106:58:57:1, with a coefficient of association of .934 corresponds approximately to a repulsion ratio with 13 percent crossing over. If the frequency of the fourth class is used as a criterion there are in both crosses 3 double recessives in the total of 474 F_2 plants, suggesting about 16 percent crossing over. The corresponding F_2 ratio obtained by Hedrick and Booth (1907) was: 252:127:70:3, indicating (Q=.843) about 20 percent crossing over (Jones, 1917). Forthcoming backcross generations should determine the precise crossover value in the range from 13 to 20 percent.

3. Pink Peach (ppP_rP_r) × Yellow Pear (PPp_rp_r). The F_1 plants, of the double dominant phenotype (PP_r), gave four classes among their 498 offspring (table 3).

Table 3

Data showing repulsion between p and p_r genes

CROSSES	F_2 PHENOTYPES			
	PP_r	Pp_r	pP_r	pp_r
Pink Peach × Yellow Pear	145	74	72	3
Yellow Pear × Pink Peach	102	51	50	1
Total	247	125	122	4

This ratio with a coefficient of association of .878 indicates a cross-over value of 17+percent, thus approximating, as theoretical considerations require, that for the previous cross. For, if peach and dwarf are completely linked, they should show equally close linkage with a third factor pair (P_r p_r).

4. Dwarf Aristocrat (*ddSS*) × Grape Cluster (*DDss*). F_1 plants from this cross were all tall with simple inflorescence, and a small F_2 generation (112 plants) segregated sharply into the expected classes: 59 tall with simple inflorescence: 23 tall with compound inflorescence: 29 dwarf simple: 1 dwarf compound. The ratio with $Q = .837$ fits into the repulsion series with 20 percent cross-overs.

The evidence presented from these four crosses would seem adequate to justify the establishment of a linkage group containing the factors: *Dd*, *Pp*, P_rp_r, and *Ss*. In all instances the linkage is close and the quantitative values within the group mutually consistent.

Crosses involving *Ss* with *Pp* and with P_rp_r and back-crosses involving all four pairs of linked genes, excepting *Dd* and *Pp*, are now in culture for the exact determination of loci and mapping of the "chromosome."

Facts are yet lacking even on such essential points as whether the loci of *Ss* and P_rp_r are on the same or opposite sides of the closely linked peach and dwarf genes, though CRANE'S findings might barely suggest as a working hypothesis that *Ss* and P_rp_r are not at all close together.[1]

Exception has been taken by LINDSTROM (1925) to admitting linkage data on the pyriform factor, p_r, since "the heredity of fruit shape is relatively complex and has not been analyzed into its component parts." Now fruit shape is undeniably a product of many coöperating factors, but it is surely justifiable to utilize genetic elements of this complex as soon as they can be identified and dissociated from the unresolved residue, as, for instance, the genes for pear shape (p_r), for irregular outline of fruit (*f*), and probably also the complementary pairs for depth (*Aa* and *Bb*), and for fasciation (*Ee* and *Ff*) described by WARREN (1925). In the first two of these cases a fairly unmistakable segregation occurs making practicable a correct unbiased classification, as we have repeatedly tested. Accordingly I have dealt with them as distinct entities.

As regards the reported complete linkage, in another group, of the factors (*Gg*) for foliage color and those assumed to control number of fruit locules (PRICE and DRINKARD, 1908, and JONES, 1917), I found that the F_2 green-foliaged and yellow-foliaged segregates have fruits with the same average locule number. Only one linkage group in the tomato may thereore, be considered established.

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[1] Backcrosses of the past summer demonstrate crossover values as follows: between *d* and p_r, 12 ± 1.5 %; between *p* and p_r, 8 ± 1.0 %; and between *d* and *p* (calculated), 4 ± 1.8 %, a value to be compared favorably with the 2.5 % observed by LINDSTROM (1926, Res. Bull. No. 93, Iowa Agric. Exp. Sta., page 104). Hence the order of genes is *d*——————*p*——————p_r——————*s* (?)

The Combinations Showing Independent Assortment

The remaining 5 pairs of factors under analysis have turned out to be inherited independently of the 4 linked genes of the first linkage group and also independently of each other in all of the 20 different combinations (not counting reciprocals) of the 9 pairs so far bred through the second generations (see table 4). These are treated as dihybrid crosses though many dihybrid ratios were extracted from trihybrid or even tetrahybrid distributions.

TABLE 4

Combinations whose F_2 ratios indicate absence (—) of detectable linkage between pairs of genes.

	Rr	*Yy*	*Gg*	*Cc*	*Ff*
Dd	—	—		—	—
Pp	—	—	—		
P_rp_r	—	—	—	—	
Rr		—		—	—
Yy				—	—
Gg	—?	—		—	—

Since different methods of calculating the goodness of fit of the observed ratios to the 9:3:3:1 ratios expected in F_2 generations produced by free assortment of F_1 genes, give somewhat different results, it has seemed best to calculate both deviations from the 10:6 ($AB+ab$:$Ab+aB$) ratio and values for X^2 and P by the PEARSON-ELDERTON method. The proofs of random dihybrid distribution afforded by the data are summarized in tables 5–10 and detailed in the descriptions of crosses which follow, and in tables 11–18 to the Appendix.

Genetic Relations between Genes *Dd* and *Rr*, *Yy*, *Cc* and *Ff*

The genetic relations existing between the factor for dwarfness and those controlling fruit flesh and skin color, leaf shape, and fruit outline are revealed in the data of table 5.

Stature and Flesh Color—Dd and *Rr* genes. Dwarf Aristocrat, a dwarf, red-fleshed variety (*ddRR*), crossed with tall yellow-fleshed sorts (*DDrr*) like Yellow Pear and Albino, gave all tall and red-fleshed F_1 hybrids, and these in turn an apparently freely segregating F_2 generation, as detailed in table 11 and summarized along with the three following combinations in table 5. The deviation from the 10:6 ratio is less than twice the probable error, while $X^2=3.734$ and $P=.297$, showing a fair probability that the 9:3:3:1 ratio correctly expresses the results observed.

Stature and Fruit Skin Color—Dd and *Yy* genes. Dwarf Aristocrat is yellow-skinned (*ddYY*), and its four separate crosses with tall clear-skinned varieties (*DDyy*) as Pink Peach, McMullen's Pink, and Albino, produced tall yellow-skinned hybrids, whose progeny were distributed in four classes with numerical frequencies fitting expectation excellently for X^2 is only 0.5134 and $P>.801$ (table 12).

Stature and Leaf Shape—Dd and *Cc* genes. Dwarf Aristocrat (*ddCC*) crossed with the tall "potato-leafed" McMULLEN's Pink (*DDcc*) resulted in tall cut-leafed hybrids whose offspring fell into four sharply defined phenotypes: 132 tall cut-leaf: 31 tall potato-leaf: 53 dwarf cut-leaf: 17 dwarf potato-leaf; these numbers accord well with the 10:6 ratio but fit only fairly well by the X^2 method. There is no sign of linkage by either method.

Stature and Fruit Contour—Dd and *Ff* genes. Dwarf Aristocrat is quite smooth fruited (*ddFF*) as compared with the Albino (*DDff*). Their tall hybrids are also relatively smooth fruited, and the two pairs of factors segregate entirely at random in the second generation, there being 119 tall smooth (*DF*): 44 tall irregular (*Df*):42 dwarf smooth (*dF*):14 dwarf irregular (*df*), an excellent fit of observation to theory.

TABLE 5

Summary of data from linkage tests between Dd, and Rr, Yy, Cc, and Ff genes.

FACTORS INVOLVED WITH *Dd*		F_2 CLASSES				TOTAL	DEVIATION FROM 10 6 RATIO	X^2	P
		DX	*Dx*	*dX*	*dx*				
Rr	observed	231	96	83	31	441	13.6± 7.0	3.734	.297
	calculated	248	82.7	82.7	27.6				
Yy	observed	519	164	175	55	913	3.4± 3.14	0.513	>.801
	calculated	513.6	171.2	171.2	57.0				
Cc	observed	132	31	53	17	233	3.4± 4.9	6.071	.110
	calculated	131.0	43.7	43.7	14.6				
Ff	observed	119	44	42	14	219	4.0± 4.9	0.460	>.801
	calculated	123.3	41	41	13.7				

[1] *Xx* stands for any factor pair in the first column.

Genetic Relations between Genes *Pp* and *Rr*, *Yy* and *Gg*

In the various "peach" crosses the factors for skin structure (pubescence) were brought into combination with those mentioned above producing contrasting fruit flesh and fruit skin colors and foliage colors. From these combinations the genes reassorted freely in each case (table 6 for summary).

Fruit Skin Pubescence and Flesh Color—Pp and *Rr* genes. The Pink Peach-Yellow Pear cross (page 392) also brought together genotypes, *ddRR* and *PPrr*, whose elements redistributed at random in an F_2 population numbering 498 plants. These contained 288 smooth red (*PR*):81 smooth yellow (*Pr*): 92 peach red (*pR*):37 peach yellow (*pr*), which ratio differs from the expected chiefly in a slight deficiency of the *r* classes.

Fruit Skin Pubescence and Skin Color—Pp and *Yy* genes. Pink Peach, a double recessive (*ppyy*), was crossed with several varieties bearing fruits with smooth yellow skins (*PPYY*), as Dwarf Aristocrat, Yellow Pear, and Honor Bright. Their hybrids bore smooth, yellow-skinned fruit, and the F_2 generation gave an almost perfect 9:3:3:1 ratio, the X^2 value being only 0.054. The 1230 plants were 689 *PY*:233 *Py*:232 *pY*:76 *py*.

Fruit Skin Pubescence and Foliage Color—Pp and *Gg* genes. Peach (*ppGG*) by Honor Bright (*PPgg*) produced green-foliaged, smooth-fruited hybrids, which segregated into 148 smooth green (*PG*):57 smooth yellow (*Pg*):53 peach Green (*pG*):16 peach yellow (*pg*) plants, X^2 is here also very low, 0.980 (table 6).

TABLE 6

Summary of data from linkage tests between Pp, and Rr, Yy, and Gg genes.

FACTORS INVOLVED WITH *Pp*		F_2 CLASSES				TOTAL	DEVIATION FROM 10.6 RATIO	X^2	*P*
		PX	*Px*	*pX*	*px*				
Rr	observed	288	81	92	37	498	13.8± 7.16	3.009	.382
	calculated	280.1	93.4	93.4	31.1				
Yy	observed	689	233	232	76	1230	3.8± 11.47	0.054	>.801
	calculated	691.9	230.6	230.6	76.9				
Gg	observed	148	57	53	16	274	7.2± 5.47	0.980	>.801
	calculated	154.1	51.4	51.4	17.1				

Genetic Relations between Genes P_rp_r and Rr, Yy, Gg and Cc

Numerous Yellow Pear crosses are the source of data, summarized in table 7, tending clearly, on the whole, to show the independence of the "pear" gene, p_r, from the genes controlling flesh and skin color, foliage color and leaf shape.

Fruit Neck Constriction and Flesh Color—P_rp_r and Rr genes. Yellow Pear (p_rp_rrr) was crossed with several varieties with the genotypic formula (P_rP_rRR), as Dwarf Aristocrat, Pink Peach, McMULLENS' Pink, and Honor Bright. The results of these crosses are detailed in table 14. The totalled F_2 classes observed were distributed in the ratio: 698 P_rR:254 P_rr:243 p_rR:80 p_rr. This obviously fits the random dihybrid ratio, X^2 equalling 1.53 and P .682.

Fruit Neck Constriction and Skin Color—P_rp_r and Yy genes. Yellow Pear (p_rp_rYY) bred with clear-skinned non-pyriform types (P_rP_ryy) like McMULLEN'S Pink and Pink Peach, produced F_2 phenotypes in the following numbers respectively: 167 P_rY:47 P_ry:62 p_rY:8 p_ry and 292 P_ry : 80 P_ry: 94 p_ry:32 p_ry. These numbers, totalled in table 7, deviate from a 10:6 ratio by 10.2 ± 9.07 and give an X^2 value $= 5.672$ with $P = .132$, the low probability being due not to association of factors but to the low frequency of both clear-skinned classes.

Fruit Neck Constriction and Foliage Color—P_rp_r and Gg genes. Yellow Pear (p_rp_rGG) on Honor Bright (P_rP_rgg) yielded an F_2 generation composed of 164 P_rG:44 P_rg:54 p_rG:17 p_rg plants, deviating little from expectation and giving a P value of .536.

Fruit Neck Constriction and Leaf Shape—P_rp_r and Cc genes. The Yellow Pear-McMULLEN'S Pink cross brought together from different parents the p_r and C genes. Their F_2 progeny fell into four distinct phenotypes, P_rC, P_rc, p_rC, and p_rc, with the ratio: 166:50:54:16, agreeing excellently with expectation (table 7).

Genetic Relations between Genes Rr and Yy, Cc and Ff

The factors for flesh color, Rr have been involved in many crosses with those for skin color, and in a few crosses with those for leaf shape and fruit contour. Summarized data from these combinations are given in table 8.

Fruit Flesh Color and Skin Color—Rr and Yy genes. These genes have been brought together in many crosses both in the repulsion phase ($RRgg \times rrYY$) and in the coupling phase ($RRYY \times rryy$). The findings are set down with details in table 15. Aside from occasional F_2 populations showing a lack of the clear-skinned classes, as noted freqeuntly in other cases, the ratios are in good agreement with the 9:3:3:1 frequencies.

Flesh Color and Leaf Shape—*Rr* and *Cc* genes. In the cross, McMullen's Pink (*RRcc*)×Yellow Pear (*rrCC*), F_1 plants have cut leaves and red fleshed fruit, and the F_2 plants fall clearly into the four expected groups. The ratio of 163 *RC*:45 *Rc*:57 *rC*:19 *rc*, deviates from the 10:6 ratio less than the probable error, and $P = .642$.

Table 7

Summary of data from linkage tests between P_rp_r, and Rr, Yy, Gg, and Cc genes

FACTORS INVOLVED WITH $P\ p_r$		F_2 CLASSES				TOTAL	DEVIATION FROM 10.6 RATIO	X^2	P
		P_rX	P_rx	p_rX	p_rx				
Rr	observed	698	254	243	80	1275	19 ± 11.76	1.529	.682
	calculated	717.2	239.0	239.0	79.7				
Yy	observed	459	127	156	40	782	10.2± 9.07	5.672	.132
	calculated	439.9	146.6	146 6	48.9				
Gg	observed	166	44	54	17	279	6.7± 5.38	2.200	.536
	calculated	156.9	52.3	52.3	17.4				
Cc	observed	166	50	54	16	286	3.2± 5.48	0.608	>.801
	calculated	160.9	53.6	53.6	17.9				

Flesh Color and Fruit Contour—*Rr* and *Ff* genes. Albino (*rrff*) bred with Dwarf Aristocrat or Honor Bright (*RRFF*) produced F_1 plants bearing red-fleshed fruits smooth and regular in outline, and these in turn split up in the following generation into types: red-fleshed, regular or irregular, and yellow-fleshed, regular or irregular. From the first cross the ratio was: 122 RF:42 Rf:39 rF:16 rf, and from the second (Alb.×H. Br):87 RF: 28 Rf: 20 rF:12 rf. The deviation from 10:6 little exceeds the probable error, and $P = .472$. (table 8).

Genetic Relations between Genes *Yy* and *Cc* and *Ff*

Fruit Skin Color and Leaf Shape—*Yy* and *Cc* genes. Hybrids between genotypes *yy cc* and *YYCC* (see table 16 for details and table 9 for summary of data) gave progeny in four classes with frequencies approximating the 9:3:3:1 ratio, if allowance be made for the deficiency of the clear-skinned terms. The deviation from a 10:6 ratio was 2.04 times the probable error.

Fuit Skin Color and Fruit Contour—*Yy* and *Ff* genes. Yellow-skinned regular-contoured fruit (*YYFF*) of varieties Dwarf Aristocrat and Honor Bright dominated over the Albino type (*yyff*). The F_2 segregation agreed well with that anticipated from a random redistribution of the two sets of factors. The two inter-variety crosses yielded respectively: 132 *YF*:40 *Yf*: 29 *yF*:18 *yf*, and 78 *YF*:32 *Yf*:29 *yF*:8 *yf*. The mathematical validity of the total numbers as agreeing with expectation is expressed in the constants (table 9).

TABLE 8

Summary of F_2 data from linkage tests between Rr, and Yy, Cc, and Ff genes.

FACTORS INVOLVED WITH *Rr*		F_2 CLASSES				TOTAL	DEVIATION FROM 10.6 RATIO	X^2	P
		RX	*Rx*	*rX*	*rx*				
Yy	observed	1026	300	326	96	1742	33.2± 13.5	6.027	.112
	calculated	979.9	326.6	326.6	108.9				
Cc	observed	163	45	57	19	284	4.5± 5.45	1.696	.642
	calculated	159.75	53.25	53.25	17.75				
Ff	observed	209	70	59	28	366	8.2± 6.16	2.554	.472
	calculated	205.9	68.6	68.6	22.9				

TABLE 9

Summary of F_2 data from linkage tests between Yy, and Cc, and Ff genes.

FACTORS INVOLVED WITH *Yy*		F_2 CLASSES				TOTAL	DEVIATION FROM 10.6 RATIO	X^2	P
		YX	*Yx*	*yX*	*yx*				
Cc	observed	433	135	117	34	719	17.6± 8.6	7.027	.072
	calculated	404.4	134.8	134.8	44.9				
Ff	observed	210	72	58	26	366	7.2± 6.04	2.307	.518
	calculated	205.9	68.6	68.6	22.9				

Genetic Relations Between Genes *Gg* and *Rr*, *Yy*, *Cc* and *Ff*

In Honor Bright crosses the factor controlling yellowing of foliage, *g*, is involved with those controlling fruit colors, leaf shapes, and fasciation of fruit. The findings are summarized in table 10.

Foliage Color and Flesh Color—Gg and *Rr* genes. Honor Bright foliage is green in new growth but soon turns yellow; and its immature fruits are very pale whitish green until they reach the ripening stage, when they assume a bright red color. The white or yellow-fleshed varieties have somewhat similar immature fruits which ripen "albino" or yellow-fleshed. In the four crosses where the *Gg* and *Rr* genes were involved segregation in F_2 plants was perfectly distinct, but difficulty was encountered because of the late ripening of some of the F_2 plants, particularly the yellow-foliaged segregates. In a few populations the classification could not be entirely completed on this account, even by bringing the fruits indoors to ripen.

Some earlier more completely classified F_2 populations appear to show weak repulsion ratios (see table 17 for details) with crossover values of about 40 percent. The later populations, less completely classified, showed a slight excess of the crossover classes, however, which nearly balances the earlier deficiency. The data cannot be considered properly critical. A back-cross generation now in culture will probably furnish the needed facts.

Foliage Color and Skin Color—Gg and *Yy* genes. With reference to these genes Honor Bright has the formula *ggYY*, and White Apple, McMullen's Pink, Golden Queen, Albino and Pink Peach are *GGyy*. Their F_2 generations fell naturally into the *GY*, *Gg*, *gy*, *gy* phenotypes (table 18). The total ratio was 557:168:168:68, deviating from a 10.6 ratio by 24.4 ± 10.0, and giving an X^2 value of 3.816 and a P value of .286. The genes are, therefore, probably not associated.

TABLE 10

Summary of F_2 data from linkage tests between Gg, and Rr, Yy, Cc, and Ff genes.

FACTORS INVOLVED WITH *Gg*		F_2 CLASSES				TOTAL	DEVIATION FROM 10 6 RATIO	X^2	P
		GX	*Gx*	*gX*	*gx*				
Rr	observed	425	151	142	31	749	12.1 ± 9.0	6 195	.104
	calculated	421.3	140.4	140.4	46.8				
Yy	observed	557	168	168	68	961	24.4 ± 10.0	3.816	.286
	calculated	540.6	180.6	180.6	60.0				
Cc	observed	121	34	42	16	213	3.9 ± 4.72	1.444	.700
	calculated	119.8	39.9	39.9	13.3				
Ff	observed	83	31	23	9	147	1.2 ± 3.94	1.210	.745
	calculated	82.7	27.6	27.6	9.2				

Foliage Color and Leaf Shape—*Gg* and *Cc* genes. Honor Bright (*ggCC*) × McMullen's Pink (*GGcc*) produced an F_2 distribution of 121 green cut-leaf (*GC*): 34 green potato-leaf (*Gc*): 42 yellow cut-leaf (*gC*): 16 yellow potato-leaf (*gc*). The deviation from the 10:6 ratio is less than the probable error, $X=1.444$ and $P=.70$.

Foliage Color and Fruit Contour—*Gg* and *Ff* genes. The F_2 product from an Honor Bright (*ggFF*) × Albino (*GGff*) cross demonstrated the independence of the genes concerned. The observed frequencies of the segregating classes: 84 *GF*: 31 *Gf*: 23 *gF*: 9 *gf*, agree very well with the theoretical frequencies (table 10).

CONCLUSIONS

From the data reported in the body of the paper and in the appendix we are clearly justified in distributing the nine pairs of genes described in six separate and independent groups, constituted as follows:

Group 1. *Dd*, *Pp*, P_rP_r, and *Ss*.
Group 2. *Rr*
Group 3. *Yy*
Group 4. *Cc*
Group 5. *Ff*
Group 6 *Gg*

These groupings must, of course, be considered as tentatively fixed bases for as many linkage groups. They are entirely consistent with all the published data reviewed and represent the most compact and logical interpretation of the facts now available.

SUMMARY

1. The first linkage group established for the tomato contains four pairs of genes, controlling respectively: vine habit (*Dd*, tall *versus* dwarf); fruit skin pubescence and structure (*Pp*, smooth *versus* "peach"); one element of the fruit shape complex (P_rp_r, non-pyriform *versus* pyriform); and type of inflorescence (*Ss*, simple *versus* "compound").

2. Within this group F_2 data indicates that *Dd* and *Pp* are completely (?) linked, and the *Dd* and P_rp_r show 13-20 percent crossing over, *Pp* and P_rp_r 17.5 percent crossing over, and *Dd* and *Ss* 20 percent crossing over. (cf. foot note to p. 393.)

3. F_2 data from 20 different combinations of genes two pairs at a time shows that (a) the genes of the first linked group are independent in inheritance from five other factor pairs controlling: fruit flesh color (*Rr*, red *versus* yellow), fruit skin color (*Yy*, yellow *versus* colorless), foliage color (*Gg*, green *versus* yellow), shape of leaf (*Cc*, cut leaf *versus* "potato leaf"), and fasciation of fruit (*Ff*, regular *versus* irregular).

(b) the five pairs of genes: *Rr*, *Yy*, *Gg*, *Cc*, and *Ff* are also independent of each other, with the possible exception of *Gg* and *Rr*.

4. The results collectively establish reasonably fixed bases for six linkage groups, which suggest in outline the genetic pattern of the tomato.

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APPENDIX—TABLES 11-13

TABLE 11

F_2 data from the cross ddRR×DDrr, showing that Dd and Rr are inherited independently.

VARIETIES CROSSED (see p 394)	*D*		*d*		TOTAL
	R	*r*	*R*	*r*	
Dw. Ar. × Y. Pr.	110	54	40	18	222
Dw. Ar. × Alb.	121	42	43	13	219
Total	231	96	83	31	441
Calculated 9:3:3:1	248	82.7	82.7	27.6	

Deviation from 10:6 ratio=13.6±6.96
$X^2=3.734$ $P= .297$

TABLE 12

F_2 data from the cross $ddYY \times DDyy$, showing that Dd and Yy are inherited independently.

VARIETIES CROSSED	*D*		*d*		TOTAL
	Y	*y*	*Y*	*y*	
Dw. Ar. × P. Pch.	125	49	44	10	228
P. Pch. × Dw. Ar.	135	52	38	15	240
Dw. Ar. × McM P.	130	29	50	17	226
Dw. Ar. × Alb.	129	34	43	13	219
Total	519	164	175	55	913
Calculated 9.3:3.1	513.6	171.2	171.2	57.0	

Deviation from 10·6 ratio=3.4±3.14
X^2= .5134 $P>$ .801

TABLE 13

F_2 data from the cross $ppyy \times PPYY$, showing that Pp and Yy are inherited independently

VARIETIES CROSSED	*P*		*p*		TOTAL
	Y	*y*	*Y*	*y*	
P. Pch. × Dw. Ar	259	99	83	27	468
P. Pch. × Y. Pr.	281	78	95	34	488
P. Pch. × H. Br	149	56	54	15	274
Total	689	233	232	76	1230
Calculated 9 3 3 1	691.9	230.6	230.6	76.9	

Deviation from 10 6 ratio=3.8±11.47
X^2= .054 $P>$ .801

TABLE 14

F^2 data from the $p_rp_rrr \times P_rP_rRR$ cross, showing that Pp and Rr are inherited independently

VARIETIES CROSSED	*P*		*p*		TOTAL
	R	*r*	*R*	*r*	
Y. Pr. × Dw. Ar	109	54	41	18	222
Y. Pr. × P. Pch.	282	90	98	28	498
Y. Pr. × McM P.	153	61	55	15	284
Y. Pr. × H. Br.	154	49	49	19	271
Total	698	254	243	80	1275
Calculated 9:3·3 1	717.2	239.0	239.0	79.7	

Deviation from 10 6 ratio=19±11.76
X^2=1.53 $P=$ .682

TABLE 15

F_2 data from the cross $RRyy \times rrYY$, indicating that Rr and Yy are inherited independently.

VARIETIES CROSSED	R		r		TOTAL
	Y	y	Y	y	
P. Pch. × Y. Pr.	300	88	86	24	498
McM. P. × Y. Pr.	170	38	60	16	284
Total	470	136	146	40	782
Calculated 9:3·3.1	439.9	146.6	146.6	48.9	

Deviation from the 10:6 ratio = 21.2 ± 8.77

$X^2 = 6.586$ $P = .088$

F_2 data from the cross $RRYY \times rryy$, showing that Rr and Yy are inherited independently.

VARIETIES CROSSED	R		r		TOTAL
	Y	y	Y	y	
Dw. Ar. × Alb.	131	33	41	14	219
H. Br. × Wh. App.	132	39	42	11	224
H. Br. × G. Q.	57	21	22	7	107
R. C. × Alb.	85	31	26	7	149
H. Br. × Alb.	88	27	22	10	147
R. C. × G. Q.	63	23	21	7	114
Total	556	174	174	56	960
Calculated 9.3:3.1	540	180	180	60	

Deviation from 10·6 ratio = 12 ± 10.04

$X^2 = 1.141$ $P = .769$

TABLE 16

F_2 data from the cross $YYCC \times yycc$, showing that Yy and Cc are independent in inheritance.

VARIETIES CROSSED	Y		y		TOTAL
	C	c	C	c	
McM. P. × Dw. Ar.	141	39	38	8	226
McM. P. × H. Br.	114	45	37	13	209
McM. P. × Y. Pr.	178	51	42	13	284
Total	433	135	117	34	719
Calculated 9:3:3·1	404.4	134.8	134.8	44.9	

Deviation from 10.6 ratio = 17.625 ± 8.62

$X^2 = 7.027$ $P = .072$

TABLE 17

F_2 data from the cross ggRR×GGrr, showing that Gg and Rr are inherited independently.

VARIETIES CROSSED	*G*		*g*		TOTAL
	R	*r*	*R*	*r*	
H. Br. × Wh. App.	123	44	48	9	224
H. Br. × G. Q.	57	24	21	5	107
H. Br. × Alb.	90	25	25	7	147
H. Br. × Y. Pr.	155	58	48	10	271
Total	425	151	142	31	749
Calculated 9:3:3:1	421.3	140.4	140.4	46.8	

Deviation from 10.6 ratio = 12.125 ± 8.97

$X^2 = 6.195$ $P = .104$

TABLE 18

F_2 data from the cross ggYY×GGyy, showing that Gg and Yy are inherited independently.

VARIETIES CROSSED	*G*		*g*		TOTAL
	Y	*y*	*Y*	*y*	
H. Br. × Wh. App	132	35	42	15	224
H. Br. × McM. P.	129	32	30	18	209
H. Br. × G. Q.	60	21	19	7	107
H. Br. × Alb.	89	26	21	11	147
H. Br. × P. Pch.	147	54	56	17	274
Total	557	168	168	68	961
Calculated 9:3:3:1	540.6	180.2	180.2	60.0	

Deviation from 10.6 ratio = 24.4 ± 10.0

$X^2 = 3.816$ $P = .286$

THE SEGREGATION OF CARBOHYDRATES IN CROSSES BETWEEN WAXY AND STARCHY TYPES OF MAIZE[1]

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The purpose of this paper is to report the Mendelian segregation of waxy and starchy carbohydrates in both the sporophytic and gametophytic generations of maize. This is of especial genetic interest because the counts of segregate waxy kernels have very commonly been short of the expected numbers, although this character appears to be a simple Mendelian recessive. COLLINS (1909) was the first to point out the physical difference in the endosperm of waxy and ordinary starchy varieties.

While the exact difference in their chemical nature has not been established, WEATHERWAX (1922) considered that the waxy carbohydrate was erythrodextrin because of its reddish color reaction to iodine as compared with the bluish staining of the endosperms of ordinary starchy varieties. DEMEREC (1924), BRINK AND MACGILLIVRAY (1924), AND LONGLEY (1924) have each established a corresponding differential staining of the pollen grains of the two maize sorts. BRINK (1925) has more recently reported finding the same chemical distinction for the carbohydrates of the embryo sacs. In a publication of earlier results the writers (1925) failed, through faulty technique, to observe the differential staining of the pollen, but later results conform with those of the above investigators. Our earlier

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observations as to the wide distribution of starch throughout the sporophytic vegetative tissues have been confirmed.

Taking into consideration the observations of all investigators, it appears that the waxy carbohydrate is restricted to haploid or 1x tissue including pollen, embryo sac, and possibly the tissue developed from the antipodals, and to the 3x tissue of the endosperm. Only ordinary starch has been found in the diploid or 2x tissues which make up the rest of the plant.

The results of this investigation as well as those of DEMEREC, BRINK AND MACGILLIVRAY, AND LONGLEY indicate that those segregated plants following hybridization which come true to endosperm type also come true to the same sort of carbohydrate in the pollen. This is evidence that a single chromosome factor controls both or else that they are very closely linked characters with no observed crossing over.

DISTINGUISHING WAXY AND STARCHY CARBOHYDRATES

Pollen

Repeated attempts to distinguish the pollen of Chinese waxy maize and its hybrids from ordinary starchy maize pollen by the iodine test during the summer of 1924, following receipt of the articles by DEMEREC, and BRINK and MACGILLIVRAY, resulted in failure so that we concluded that such a difference did not exist. Further tests during 1925 showed that with proper staining it is easy to distinguish the two types of pollen and show the segregation in hybrids.

The following method was used succesfully: The pollen was placed on a slide with a few drops of stock solution of iodine in potassium iodide[1] diluted with 3 parts of distilled water. The slide was then left uncovered, more iodine solution being added if necessary, until the pollen appeared sufficiently stained. A small crystal of chloral hydrate was then added as a clearing agent. When this was dissolved the cover glass was applied. The pollen should all be moistened at the same time in order to stain each type of pollen uniformly.

By this method the carbohydrate grains of the pollen of ordinary starchy maize are stained a deep blue, while those in waxy maize are stained red, leaving other parts of the pollen grains unstained and cleared. When pollen is stained in this manner, any imperfect pollen grains which lack carbohydrate reserves remain unstained and may be distinguished from the normal pollen. These methods apply to pollen stored dry as well as in the fresh condition.

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[1] The stock solution was made by dissolving 1 gram of potassium iodide and 0.3 gram iodine in 100 cc. distilled water.

Endosperm

Satisfactory differential staining of the carbohydrates in the endosperm is more easily secured than in the case of the pollen, due, perhaps, to the much larger starch grains in the endosperm. (Figures 1b and 1c). If too strong iodine solution is used, there may be a tendency to produce such an intense stain that the endosperms all appear black rather than red or blue. The ordinary starchy endosperm seems to have a greater affinity for the iodine and will stain in a weaker solution than the waxy.

For extensive observations, rapid results may be secured by dipping the ears into iodine solution after the endosperms have been exposed by cutting off the crowns from the kernels. This may be done easily before the grain has hardened. The ears stain more promptly and evenly when first dipped momentarily in boiling water. After staining, the ears may be rinsed with tap water to remove loose starch which might cause confusion. Should the color fade before completing the counts, the ear may be restained.

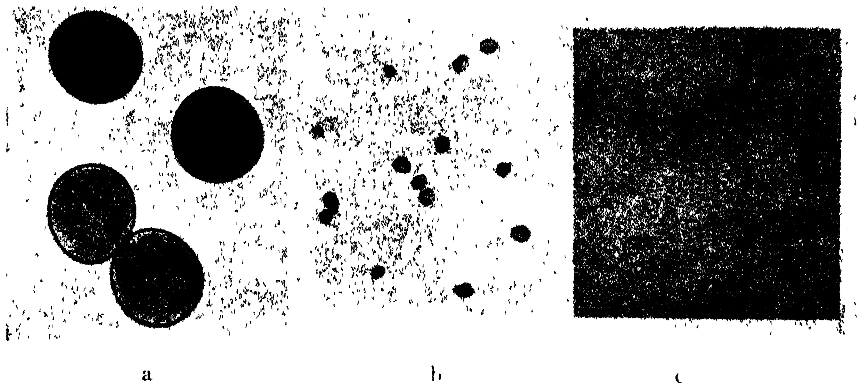

Figure 1a. Representative pollen grains from a heterozygous plant showing segregation into equal numbers of waxy and starchy grains which stain red and blue respectively, with iodine. Those which stain red and carry the factor for waxy are shown light in this photograph.

Figure 1b. Typical carbohydrate grains from the endosperm of maize, to be compared for size with those from pollen, shown in figure 1c.

Figure 1c. Typical carbohydrate grains from pollen to be compared for size with those from the endosperm of maize, shown in figure 1b.

Prior to the discovery of the difference in staining with iodine in 1922, all separations of starchy and waxy kernels were necessarily based on their difference in physical appearance. This can be done fairly accurately when maize is thoroughly cured. A dark-colored aleurone layer which is frequently present in crosses with Chinese maize tends to obscure the difference and makes such separation more difficult. If the starchy segregates are of a "soft flour" type, the distinction is far less certain than in the case of more horny texture.

TABLE 1

Pollen segregation in hybrids of waxy×starchy maize, as determined by the reaction to iodine.

TASSEL NO.	NUMBER STAINING			RATIO RED TO BLUE	TASSEL NO.	NUMBER STAINING			RATIO RED TO BLUE
	Blue	Red	No stain			Blue	Red	No stain	
F_1 Reid Yellow Dent×Chinese									
1	125	124	2	1.00	6	193	152	3	0.79
2	94	92	.	0.99	7	118	112		0.95
3	131	156	5	1.19	8	210	217	2	1.03
4	102	137	2	1.34	9	289	271	1	0.95
5	175	189	4	1.08	10	294	314	..	1.06
					Total	1731	1764	19	1.02
F_1 Hogue Yellow Dent×Chinese									
11	314	354	2	1.13	14	302	296	4	0.93
12	356	335	7	0.94	15	324	358	2	1.10
13	355	369	3	1.04	16	334	296	1	0.89
					Total	1985	2008	19	1.01
F_2 Chinese×Yellow Flint									
17	295	292	..	0.99	37	167	184	6	1.10
18	128	157		1.23	38	308	310	8	1.01
19	340	284		0.84	39	341	346	2	1.01
20	321	279		0.87	40	73	94		1.29
21	190	198		1.04	41	264	269	.	1.02
22	424	493		1.16	42	109	109	.	1.00
23	191	180	6	0.94	43	186	170	.	0.91
24	139	122	5	0.88	44	143	129	5	0.90
25	336	315	2	0.94	45	75	57	.	0.76
26	225	229	8	1.02	46	56	55	.	0.98
27	242	327	21	1.35	47	196	215	6	1.10
28	201	252	..	1.25	48	267	323	1	1.21
29	424	428	.	1.01	49	299	301	9	1.01
30	207	191	7	0.92	50	748	734	.	0.98
31	112	116		1.04	51	236	257	..	1.09
32	201	206		1.02	52	296	308	..	1.04
33	35	43	63	1.23	53	384	378	14	0.98
34	28	25	.	0.89	54	290	261	3	0.90
35	347	392	21	1.13	55	41	45	2	1.10
36	118	85	36	0.72	56	315	298	..	0.95
					Total	9298	9457	225	1.02

SEGREGATION OF POLLEN

Counts were made for the pollen from 88 segregating plants, resulting in 13014 pollen grains stained blue and 13229 stained red. This is 49.59 percent blue and 50.41 percent red. The imperfect grains which often appear have not been included in calculating the ratios. The results are given in table 1. The variations from the 1:1 ratio are doubtless due mainly to the use of samples which were not exactly representative rather than to actual differences in number of the two types of pollen. This statement is confirmed by the lack of correlation between pollen and kernel ratios as shown in table 2.

Demerec (1924), and Brink and MacGillivray (1924) reported that approximately equal numbers of the two types of pollen grains were found in plants heterozygous for the waxy character. Longley (1924) has also reported such segregation in the pollen of crosses of waxy with starchy maize and teosinte, and in crosses between starchy and waxy Coix. Parnell had previously reported segregation of the pollen in crosses between glutinous and ordinary rice.

SEGREGATION OF ENDOSPERM

A large number of ears were hand-pollinated with segregating pollen. These belong to 3 groups: (1) pure Chinese ears pollinated with F_1 waxy × starchy pollen; (2) F_1 waxy × starchy plants selfed; and (3) F_2 plants grown from starchy segregated seed, selfed. In the pure Chinese fertilized by F_1 pollen 50 percent waxy kernels would be expected while in the case of F_1 and F_2 ears, the expectation would be 25 percent waxy kernels.

The ear shoots were bagged sufficiently in advance of silking to insure against contamination by stray viable pollen. As an additional precaution the pure Chinese maize was grown in isolation and detasseled because this variety tends to silk while the shoots are small and still enclosed within the leaf sheath.

Segregation where only 1 parent is heterozygous.

In this test pure waxy maize was fertilized by pollen from F_1 starchy × waxy plants. Since the ovules all carried the recessive waxy factor, the ratio of the two types of pollen would determine the kernel segregation unless influenced by differential fertilization.

Sixty-six ears of this combination were obtained as reported in table 2. These ears had been fertilized by pollen from 9 F_1 Reid × Chinese plants whose pollen segregation had been established by the iodine stain. Of

18549 kernels produced 51.5 percent proved to be waxy as determined mainly by the iodine test.

TABLE 2

Endosperm segregation of pure waxy (Chinese) maize fertilized by segregating pollen from F_1, waxy×starchy (Reid×Chinese) plants.

Ear No.	Number of kernels			Percent waxy kernels	Percent deviation of waxy	Dev.	Pollen	
	Total	Waxy	Expected waxy			P.E †	From tassel No. (Table 1)	Percent staining red*
1	343	180	171	53.5	3.5	1.44	1	49.8
2	386	186	193	48.2	−1.8	1.06	1	49.8
3	291	152	146	52.2	2.2	1.04	2	49.5
4	209	115	104	55.0	5.0	2.26	2	49.5
5	301	166	151	55.1	5.1	2.56	2	49.5
6	143	74	71	51.8	1.8	0.74	2	49.5
7	55	21	28	38.2	−11.8	3.11	2	49.5
8	127	69	63	54.3	4.3	1.58	2	49.5
9	222	98	111	44.1	−5.9	2.59	3	54.4
10	432	236	216	54.6	4.6	2.85	3	54.4
11	410	229	205	55.9	5.9	3.51	3	54.4
12	325	151	163	46.5	−3.5	1.97	3	54.4
13	398	229	199	57.5	7.5	4.46	3	54.4
14	348	182	174	52.3	2.3	1.27	3	54.4
15	306	171	153	55.9	5.9	3.05	4	57.3
16	120	67	60	55.8	5.8	1.90	4	57.3
17	273	121	137	44.3	−5.7	2.87	4	57.3
18	345	155	172	44.9	−5.1	2.72	4	57.3
19	420	223	210	53.1	3.1	1.88	4	57.3
20	389	175	195	45.0	−5.0	3.01	4	57.3
21	427	249	213	58.3	8.3	5.16	4	57.3
22	193	98	97	50.8	0.8	....	4	57.3
23	95	52	47	54.7	4.7	1.52	5	51.9
24	317	159	158	50.2	0.2	0.16	5	51.9
25	105	52	53	49.5	−0.5	0.29	5	51.9
26	160	73	80	45.6	−4.4	1.64	5	51.9
27	94	50	47	53.2	3.2	0.92	5	51.9
28	105	56	52	53.3	3.3	1.16	5	51.9
29	276	151	138	54.7	4.7	2.32	5	51.9
30	163	96	82	58.9	8.9	3.25	5	51.9
31	459	241	229	52.5	2.5	1.64	5	51.9
32	332	162	166	48.8	−1.2	0.65	5	51.9
33	272	160	136	58.8	8.8	4.32	5	51.9
34	241	130	121	54.5	4.5	1.71	5	51.9
35	254	143	127	56.3	6.3	2.98	5	51.9
36	346	188	173	54.3	4.3	2.39	5	51.9
37	323	199	162	61.6	11.6	4.46	5	51.9
38	366	182	183	49.7	−0.3	0.15	5	51.9
39	401	192	201	47.9	−2.1	1.33	5	51.9
40	456	196	228	43.0	−7.0	4.44	5	51.9
41	448	208	224	46.4	−3.6	2.24	5	51.9
42	339	165	170	48.7	−1.3	0.81	6	44.1

TABLE 2 (continued)

Endosperm segregation of pure waxy (Chinese) maize fertilized by segregating pollen from F_1, waxy×starchy (Reid×Chinese) plants.

EAR No.	NUMBER OF KERNELS			PERCENT WAXY KERNELS	PERCENT DEVIATION OF WAXY	DEV.	POLLEN	
	Total	Waxy	Expected waxy			P.E.†	From tassel No. (Table 1)	Percent staining red*
43	348	184	174	52.9	2.9	1.59	6	44.1
44	286	133	143	46.5	−3.5	1.75	6	44.1
45	357	166	178	46.5	−3.5	1.88	6	44.1
46	360	171	180	47.5	−2.5	1.41	6	44.1
47	251	133	125	53.0	3.0	1.27	7	48.7
48	305	142	153	46.6	−3.4	1.87	7	48.7
49	335	172	167	51.3	1.3	0.81	7	48.7
50	428	193	214	45.1	−4.9	3.01	7	48.7
51	190	107	95	56.3	6.3	2.58	9	48.4
52	261	145	131	55.6	5.6	2.57	9	48.4
53	205	113	102	55.1	5.1	2.28	9	48.4
54	412	214	206	51.9	1.9	1.17	9	48.4
55	325	164	163	50.5	0.5	0.16	9	48.4
56	425	250	212	58.8	8.8	5.47	9	48.4
57	273	143	137	52.4	2.4	1.08	10	51.6
58	65	37	32	56.9	6.9	1.84	10	51.6
59	78	43	39	55.1	5.1	1.34	10	51.6
60	232	126	116	54.3	4.3	1.95	10	51.6
61	215	113	108	52.6	2.6	1.01	10	51.6
62	81	39	40	48.2	−1.8	3.29	10	51.6
63	243	130	122	53.5	3.5	1.52	10	51.6
64	171	86	85	50.3	0.3	2.27	10	51.6
65	342	175	171	51.2	1.2	0.64	10	51.6
66	346	166	173	48.0	−2.0	1.11	10	51.6
Total	18549	9547	9275	51.5	1.5	5.92		
Ears grouped according to source of pollen								
1–2	729	368	365	50.5	0.5	0.33	1	49.8
3–8	1126	597	563	53.0	3.0	3.00	2	49.5
9–14	2135	1125	1068	52.7	2.7	3.66	3	54.4
15–22	2473	1259	1236	50.9	0.9	1.37	4	57.3
23–41	5213	2690	2606	51.6	1.6	2.45	5	51.9
42–46	1690	819	845	48.5	−1.5	1.88	6	44.4
47–50	1319	640	660	48.5	−1.5	1.63	7	48.7
51–56	1818	993	909	54.6	4.6	5.85	9	48.4
57–66	2046	1058	1023	51.7	1.7	2.30	10	51.6
1–66	18549	9547	9275	51.5	1.5	5.92		50.5

†Probable errors were calculated by the usual formula: $.6745\sqrt{P\times Q} \times \sqrt{\text{Total number}}$

*Imperfect pollen grains which failed to stain were not included in the percentage calculation.

In comparison with these results (table 3) in which the male parent was segregating, KEMPTON (1919) found an average shortage of 0.8 percent for the waxy kernels although one combination involving 8791 kernels showed no deficiency. Data reported by BREGGER (1918) in connection with linkage studies show a one-percent shortage from the expected number of waxy kernels. With 33020 kernels, BRINK (1925) shows a deficiency of 1.7 percent. Summarizing all of the results, there are 203 ears with 79,381 kernels of which 49.3 percent are waxy.

TABLE 3

Historical summary showing the endosperm segregation in pure waxy maize fertilized by segregating pollen from waxy×starchy hybrids. The expected ratio is 50 percent waxy kernels.

INVESTIGATORS AND THEIR TABLE NUMBERS	NUMBER OF EARS	NUMBER OF KERNELS			PERCENT WAXY	PERCENT DEVIATION OF WAXY	DEV. / P.E.	PERCENT OF EARS BELOW 50% WAXY
		Total	Waxy	Expected waxy				
KEMPTON (1919) Table 3	27	11,724	5,802	5,862	49.6	−0.4	1.65	56
KEMPTON (1919) Table 6	20	8,791	4,397	4,396	50.0	0.0	0.00	45
KEMPTON (1919) Table 10	11	6,138	2,925	3,069	47.7	−2.3	5.44	73
Total—KEMPTON	58	26,653	13,124	13,327	49.2	−0.8	3.69	55
BREGGER (1918)	4	1,159	568	580	49.0	−1.0	1.05	50
BRINK (1925) Tables 15–16	75	33,020	15,934	16,510	48.3	−1.7	9.38	67
KIESSELBACH AND PETERSEN (1926) Table 2	66	18,549	9,547	9,275	51.5	1.5	5.92	33
Total	203	79,381	39,173	39,690	49.3	−0.7	5.44	52

In reverse combinations in which the female was the segregating parent, 5 investigators (table 4) report segregations which total a deficiency of 0.02 percent from the expected percentage of waxy kernels. These tests represent a total of 198 ears and 90,944 kernels.

Segregation where both parents are heterozygous.

In these tests both F_1 and F_2 hybrids were included. Since half of the ovules carry the dominant starchy factor, the kernels which result from them will be starchy regardless of the type of pollen. The ratio of the waxy and starchy kernels produced from the other half will be determined by the ratio of the 2 types of functioning pollen grains. Thus approximately a 3:1 ratio is expected in these combinations. The ears of two varietal F_1 crosses, Reid × Chinese and Chinese × Hogue were selfed for an investigation of kernel segregation. A total of 39 selfed ears were obtained, 23 of the former and 16 of the latter cross (table 5). Of a total of 12,376 kernels in the Reid × Chinese cross, 23.2 percent were waxy, while 25.1 percent of the 8,908 Chinese × Hogue kernels were waxy. Twenty-four percent of all the kernels from both combinations were waxy. In another test starchy segregate seed from selfed F_1 ears of a Chinese × Flint cross were planted for a further study of segregation in the F_2 generation. Of the 101 selfed ears produced, 68 were found to be segregating. This corresponds almost exactly with the expected 2:1 ratio. Of the 19,126 kernels (table 6) produced on these segregating ears, 24.2 percent were of the waxy type.

TABLE 4

Historical summary showing the endosperm segregation in heterozygous ears from waxy ×starchy hybrids fertilized by pollen from pure waxy maize. The expected ratio is 50 percent waxy kernels.

INVESTIGATORS AND THEIR TABLE NUMBERS	NUMBER OF EARS	NUMBER OF KERNELS			PERCENT WAXY	PERCENT DEVIATION OF WAXY	DEV. / P E	PERCENT OF EARS BELOW 50% WAXY
		Total	Waxy	Expected waxy				
COLLINS and KEMPTON (1911) Table 2	2	1,228	526	614	42.8	-7.2	7.5	100
KEMPTON (1919) Table 4	39	15,305	7,817	7,653	51.0	1.0	3.93	31
KEMPTON (1919) Table 7	26	13,027	6,447	6,514	49.5	0.5	1.71	62
KEMPTON (1919) Table 11	8	2,866	1,451	1,433	50.7	0.7	1.00	38
Total—COLLINS and KEMPTON, and KEMPTON	75	32,426	16,241	16,213	50.1	0.1	..	80
HUTCHISON Tables 4, 5, 6, and 7	35	19,032	9,583	9,516	50.4	1.4	1.55	46
BREGGER (1918)	6	2,437	1,173	1,219	48.1	−1.9	2.76	67
BRINK (1925) Tables 13, 14	82	37,049	18,457	18,525	49.8	−0.2	1.05	56
Total	198	90,944	45,454	45,472	49.98	−0.02	0.18	50

TABLE 5

Endosperm segregation in selfed F_1 waxy×starchy maize hybrids.

EAR NO	NUMBER OF KERNELS			PERCENT WAXY	PERCENT DEVIATION OF WAXY	DEV. / P.E.	POLLEN FROM TASSEL NO. (Table 1)
	Total	Waxy	Expected waxy				
		Reid Yellow Dent×Chinese					
1	502	141	126	28.1	3.1	2.29	*
2	609	137	152	22.5	−2.5	2.08	*
3	765	170	191	22.2	−2.8	2.60	*
4	726	177	182	24.4	−0.6	0.64	*
5	638	146	159	22.9	−2.1	1.76	*
6	802	181	201	22.6	−2.4	2.42	1
7	734	172	183	23.4	−1.6	1.39	2
8	22	3	5	13.6	−11.4	1.47	3
9	490	112	122	22.9	−2.1	1.55	*
10	571	133	143	23.3	−1.7	1.43	4
11	879	235	220	26.7	1.7	1.73	5
12	240	51	60	21.3	−3.7	1.99	7
13	140	42	35	30.0	5.0	2.03	8
14	597	143	149	24.0	−1.0	0.84	9
15	304	78	76	25.7	0.7	0.39	*
16	263	68	66	25.9	0.9	0.42	*
17†	762	172	190	22.6	−2.4	2.23	10
18	337	75	84	22.3	−2.7	1.68	*
19	464	84	116	18.1	−6.9	5.09	*
20	671	135	168	20.1	−4.9	4.37	*
21	695	157	174	22.6	−2.4	2.21	*
22	602	131	151	21.8	−3.2	2.79	*
23	563	124	141	22.0	−3.0	2.45	*
Total	12376	2868	3094	23.2	−1.8	6.93	
		Chinese×Hogue Yellow Dent					
24	592	147	148	24.8	−0.2	0.14	*
25	596	140	149	23.5	−1.5	1.26	*
26	575	136	144	23.7	−1.3	1.14	*
27	583	152	146	26.2	1.2	0.85	*
28	668	180	167	26.9	1.9	1.72	*
29	789	212	197	26.9	1.9	1.83	*
30	191	47	48	24.6	−0.4	0.25	11
31	579	165	145	28.5	3.5	2.84	*
32	428	102	107	23.8	−1.2	0.83	12
33	646	180	162	27.9	2.9	2.43	*
34	362	113	91	31.2	6.2	3.96	*
35	86	49	72	17.1	−7.9	4.65	*
36	605	136	151	22.5	−2.5	2.09	*
37	808	195	202	24.1	−0.9	0.98	*
38	637	140	159	22.0	−3.0	2.59	*
39	563	139	141	24.7	−0.1	0.29	*
Total	8908	2233	2227	25.1	0.1	0.22	
Total of both crosses	21284	5101	5321	24.0	−1.0	5.14	

*Where tassel numbers are omitted the pollen had not been examined

†The bracket indicates that the two ears were borne on the same plant.

TABLE 6

Endosperm segregation in selfed F_2 plants heterozygous for the waxy character in a waxy×starchy (Chinese×Yellow Flint) hybrid.

EAR No.	NUMBER OF KERNELS			PERCENT WAXY KERNELS	PERCENT DEVIATION OF WAXY	DEV. / P.E.	POLLEN FROM TASSEL No. (Table 7)
	Total	Waxy	Expected waxy				
1	312	60	78	19.2	−5.8	3.49	*
2†	295	67	74	22.7	−2.3	1.39	14
3	288	63	72	21.9	−3.1	1.83	14
4	328	89	82	27.4	2.4	1.32	18
5	313	78	78	24.9	−0.1	0.19	*
6	296	79	74	26.7	1.7	1.00	19
7	127	31	32	24.4	−0.6	0.30	*
8	120	24	30	20.0	−5.0	1.86	*
9	224	56	56	25.0	0.0	.	22
10	328	82	82	25.0	0.0	. . .	*
11	270	56	68	20.7	−4.3	2.52	24
12	251	66	63	26.3	1.3	0.65	24
13	417	89	104	21.3	−3.7	2.68	25
14	118	30	30	25.4	0.4		26
15	305	80	76	26.2	1.2	0.78	27
16	158	35	40	22.2	−2.8	1.63	28
17	346	80	86	23.1	−1.9	1.10	29
18	209	58	52	27.8	2.8	1.42	32
19	151	39	38	25.8	0.8	0.28	33
20	463	128	116	27.7	2.7	1.91	34
21	303	79	76	26.1	1.1	0.59	35
22	301	72	75	23.9	−1.1	0.59	37
23	379	105	95	27.7	2.7	1.76	38
24	347	84	87	24.2	−0.8	0.55	39
25	126	26	32	20.6	−4.4	1.83	42
26	74	19	18	25.7	0.7	0.40	47
27	202	56	51	27.7	2.7	1.20	50
28	80	19	20	23.8	−1.2	0.38	55
29	393	99	98	25.2	0.2	1.72	*
30	193	49	48	25.4	0.4	0.25	*
31	345	83	86	24.1	−0.9	0.55	*
32	219	49	55	22.4	−2.6	1.39	*
33	367	98	92	26.7	1.7	1.07	*
34	243	52	61	21.4	−3.6	1.98	*
35	377	89	94	23.6	−1.4	1.06	*
36	200	49	50	24.3	−0.7	0.24	*
37	337	93	84	27.6	2.6	1.68	*
38	219	58	55	26.5	1.5	0.69	*
39	66	25	17	37.9	12.9	3.38	*
40	337	82	84	24.3	−0.7	0.37	*
41	357	87	89	24.4	−0.6	0.36	*
42	117	35	29	29.9	4.9	1.90	*

*Where tassel numbers are omitted the pollen had not been examined.

†The bracket indicates that the two ears were borne on the same plant.

TABLE 6 (continued)

Endosperm segregation in selfed F_2 plants heterozygous for the waxy character in a waxy×starchy (Chinese×Yellow Flint) hybrid.

EAR NO	NUMBER OF KERNELS			PERCENT WAXY KERNELS	PERCENT DEVIATION OF WAXY	DEV / P E	POLLEN FROM TASSEL NO (Table 7)
	Total	Waxy	Expected waxy				
43	421	97	105	23.0	−2.0	1.82	44
44	312	72	78	23.1	−1.9	1.16	48
45	400	108	100	27.0	2.0	1.37	*
46	188	48	47	25.5	0.5	0.25	*
47	236	48	59	20.3	−4.7	2.84	*
48	357	89	89	24.9	−0.1	..	*
49	354	92	89	26.0	1.0	0.55	*
50	292	69	73	23.6	−1.4	1.60	*
{51	342	66	85	19.3	−5.7	4.26	*
52}	315	60	79	19.0	−6.0	3.67	*
{53	479	114	120	23.8	−1.2	1.25	*
54}	370	77	92	20.8	−4.2	3.38	*
55	206	38	52	18.4	−6.6	3.34	*
56	172	38	43	22.1	−2.9	0.31	*
57	214	46	53	21.5	−3.5	1.64	*
58	44	12	11	27.3	2.3	0.52	51
59	249	52	62	20.9	−4.1	2.17	*
60	349	86	87	24.6	−0.4	0.37	*
61	389	72	97	18.5	−6.5	4.34	*
62	475	115	119	24.2	−0.8	0.63	*
63	362	94	90	26.0	1.0	0.72	*
64	538	129	135	24.0	−1.0	0.98	*
65	381	130	95	34.1	9.1	6.14	*
66	264	72	66	27.3	2.3	1.26	*
67	208	34	52	16.4	−8.6	4.28	*
68	308	67	77	21.8	−3.2	1.95	*
Total	19126	4623	4781	24.2	−0.8	3.91	

*Where tassel numbers are omitted, the pollen had not been examined.

†The bracket indicates that the two ears were borne on the same plant.

A summary of the results (table 7) obtained by COLLINS and KEMPTON (1911), and KEMPTON (1919) from crosses corresponding with the above in that both parents were heterozygous show that of 102,429 kernels 23.9 percent were waxy. In observations by BRINK (1925) with 10,032 kernels, 22.9 percent were of the waxy type. Summarizing all data where both parents were heterozygous, 356 ears including 152,871 kernels resulted in 23.9 percent waxy kernels.

TABLE 7

Historical summary showing the endosperm segregation where both parents are heterozygous for the waxy character. The expected ratio is 25 percent waxy kernels

INVESTIGATORS AND THEIR TABLE NUMBERS	NO. OF EARS	NUMBER OF KERNELS			PERCENT WAXY	PERCENT DEVIATION OF WAXY	DEV. / P.E.	PERCENT OF EARS BELOW 25 % WAXY
		Total	Waxy	Expected waxy				
COLLINS and KEMPTON (1911) Table 1	45	22,339	5,179	5,585	23.1	-1.9	9.24	64
KEMPTON (1919) Table 1	96	54,759	13,088	13,690	23.9	-1.1	8.8	65
KEMPTON (1919) Table 4	28	12,474	3,114	3,118	25.0	0.0	0.1	54
KEMPTON (1919) Table 7	17	8,177	1,981	2,044	24.3	-0.7	2.4	71
KEMPTON (1919) Table 9	4	1,055	257	264	24.3	-0.7	0.74	50
KEMPTON (1919) Table 11	8	3,625	881	906	24.3	-0.7	1.42	62
Total, COLLINS and KEMPTON, and KEMPTON	198	102,429	24,500	25,607	23.9	-1.1	11.85	64
BRINK* (1925) Table 7	15	1,263	258	316	20.4	-4.6	5.59	80
BRINK, Table 8	13	3,569	855	892	24.0	-1.0	2.13	69
BRINK, Table 9	6	1,675	384	419	22.9	-2.1	2.93	100
BRINK, Table 10	7	1,717	399	429	23.2	1.8	2.48	86
BRINK, Table 11	4	869	209	217	24.1	-0.9	0.92	50
BRINK, Table 12	6	939	195	235	20.8	-4.2	4.47	67
Total, BRINK	51	10,032	2,300	2,508	22.9	-2.1	7.11	78
KIESSELBACH and PETERSEN (1926) Table 5	39	21,284	5,101	5,321	24.0	-1.0	5.14	67
Same, Table 6	68	19,126	4,623	4,782	24.2	-0.8	3.91	59
Total, KIESSELBACH and PETERSEN	107	40,310	9,723	10,078	24.1	-0.9	6.06	63
Total all investigators	356	152,871	36,523	38,218	23.9	-1.1	14.85	65

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*In table 7 the silks were allowed to grow extra long before pollination, while in table 8 normal silks were used for comparison. In table 9, 10, and 11, the pollen had been treated with ultraviolet light, and in table 12, the pollen had been desiccated before use.

DEVIATION FROM EXPECTED RATIOS

Several explanations have been offered to account for the deviations from expected ratios noted throughout the foregoing investigations. The totals for most groups of ears fertilized by segregating pollen have shown such consistent deficiency of waxy kernels as to appear significant. The suggestions by KEMPTON (1919) to account for the deficiency may be summarized as follows:

(1) The two types of gametes may form in unequal numbers.

(2) There may be a differential death rate of the gametes during their formation so that equal numbers do not function.

(3) Lack of vigor of the pollen carrying the factor for waxy causing slower growth of their pollen tubes.

(4) Differential selection of sperms by female gametes.

(5) Greater death rate of zygotes homozygous for waxy.

(6) Mistakes of kernel classification.

After the discovery of the difference in the chemical nature of the carbohydrate reserves of the pollen, BRINK and MACGILLIVRAY (1924) suggested that the deficiency of waxy kernels may be associated with a difference in the metabolism of the pollen leading to a slower growth of the pollen tubes carrying the waxy factor.

BRINK (1925) has indicated that there may be other factors operating in conjunction with waxy which causes a deviation in the number of waxy kernels by influencing the rate of pollen tube growth. Such a factor resulting in a deviation in the number of sweet kernels has been reported by JONES and MANGELSDORF (1925). WENTZ (1925) has also presented a case which may be accounted for by such factors.

In addition to the above possible explanations the following are offered:

(1) Differential retention of viability by pollen under the artificial conditions frequently accompanying control pollination. BRINK'S experiment in which the deficiency of waxy kernels was increased by desiccating the pollen over calcium chloride suggest differential viability in stored pollen.

(2) Accidental contamination by stray pollen.

(3) Since the starchy outweighed the waxy in paired segregating kernels on a number of ears examined, it would seem that the starchy pollen may also have a greater quantity of carbohydrate reserve, resulting in differential pollen tube growth.

CONCLUSIONS

An examination of all available data leads to the following conclusions:

The endosperm of certain maize has come to be known as *waxy* because of its peculiar appearance which distinguishes it from the endosperms of other types of maize. This is due to the presence of a carbohydrate which stains red with iodine, while ordinary starch stains blue.

This term, *waxy*, has been extended for convenience to this carbohydrate wherever found in other parts of the plants, and to the factor which determines it.

Waxy carbohydrate occurs in the pollen and embryo sacs as well as endosperms of waxy maize and so far as known is limited to these regions. The presence of this carbohydrate in these three regions appears to be determined by the same factor.

In crosses with starchy maize this waxy character behaves as a simple Mendelian recessive. The hybrid kernels have starchy endosperms. When planted no waxy carbohydrate appears in the plant until after the reduction division when equal numbers of gametophytes with the starchy or waxy carbohydrate (pollen and embryo sacs) are found as shown by the iodine test.

Kernel segregation for waxy endosperm occurs on ears produced by (1) homozygous waxy plants fertilized by segregating pollen, (2) plants heterozygous for waxy, fertilized by pure waxy pollen, and (3) plants heterozygous for waxy fertilized by segregating pollen.

In the first two combinations 50 percent waxy kernels would be expected and 25 percent waxy in the last case, if free from differential fertilization. However, the combined results of all investigators for those combinations in which segregating pollen was used show a slight deficiency of waxy kernels.

Of the various explanations that have been offered for this deficiency the most plausible would seem to be, (1) unequal fertilization resulting from differential pollen tube growth dependent upon the waxy factor or a linked factor, and (2) systematic errors connected with either the pollinations or with the kernel classification.

The theory of such differential pollen tube growth is supported primarily by the fact that when all results are combined, there is a deficiency of 0.7 percent waxy kernels from the expected 50 percent ratio and 1.1 percent deficiency from the expected 25 percent ratio when segregating pollen was used, whereas no deviation from expected ratios resulted when pure waxy pollen and segregating ovules were used. The greater deficiency secured by BRINK with extra long silks tends further to substantiate this.

Based on probabilities the deviation from the expected ratio, if due to slower pollen tube growth associated with the waxy factor, should be twice as large when only the male parent is segregating as when both parents segregate. This is contrary to the results stated above and suggests that other causes are involved. This is further indicated by the fact that only 60 percent of the total combined number of 559 ears fertilized by segregating pollen showed a deficiency of waxy kernels, and also by the fact that certain rather large groups of such ears were free from deficiency.

The evidence at hand seems insufficient to definitely establish the causes or the significance of the deviations herein reviewed.

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THE EXPRESSION OF MENDELIAN FACTORS IN THE GAMETOPHYTE OF MAIZE

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DISTORTED RATIOS IN DEFECTIVE SEEDS

In 1918 a number of plants of a large-seeded, yellow, variety of flint corn, known as Gold Nugget, were self-pollinated. Nineteen selfed ears were secured and among these were three which segregated for a type of endosperm abnormality to which has been applied (JONES 1920) the term "defective seeds". Defective seeds may be described as lethal or semi-lethal characters in which the endosperm and embryo are greatly reduced in size or almost completely lacking. These characters appear very frequently when maize is inbred and it has been shown (MANGELSDORF 1923, 1926) that there are many genetically distinct types of this abnormality.

The defective which appeared in the Gold Nugget variety and to which the factor symbol dc_1 has been given, is a semi-lethal type. The recessive seeds are characterized by a smaller size and aborted, shrivelled appearance. Their development is about half that of normal seeds on the same ears and they can, in most cases, be readily and accurately separated from the latter.

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[1] Also contribution from the BUSSEY INSTITUTION of HARVARD UNIVERSITY.

In 1920 the strain carrying the de_1 factor was crossed with a defective from another variety. In the F_2 endosperm generation of this cross, three ears segregating for defective seeds of the de_1 type were obtained. These ears bore 567 normal and 194 defective seeds, almost a perfect 3:1 ratio. The natural conclusion was that this character is inherited as a simple Mendelian recessive.

In 1922 a de_1 strain of slightly different pedigree from the one used in 1920, but closely related to the latter, served as a pollen parent in crosses with five different unrelated stocks. In the F_2 generation of these crosses fourteen progenies segregating for the parental de_1 type were recovered.

TABLE 1

Progenies segregating for de_1 in the F_2 endosperm generation of crosses made in 1922.

EAR NO.	NORMAL SEEDS	DEFECTIVE SEEDS	DEVIATION	$\frac{\text{DEVIATION}}{\text{PE}}$	PERCENT DEFECTIVE
22	160	49	−3	.7	23.4
24	154	63	9	2.1	29.0
25	183	90	22	4.6	33.0
42	184	55	−5	1.2	23.0
126	428	126	−15	2.2	22.7
128	265	90	1	.2	25.4
129	369	180	43	6.3	32.8
173	200	93	20	4.0	31.7
388	498	197	23	3.0	28.3
389	424	155	6	9	26.8
391	127	61	14	3.5	32.4
392	328	82	−21	3.6	20.0
393	284	97	2	.3	25.5
1520	346	175	45	6.8	33.6
Total	3950	1513	147	6.8	27.7

When the counts of normal and defective seeds on these ears were combined (table 1) it was found that the defectives occurred in excess of the theoretical expectation. The average percentage of defectives in this group was 27.7. This represents a deviation from a 3:1 ratio of 6.8 times the probable error and is undoubtedly significant. A deviation of this size should occur, by chance alone, only once in several hundred thousand trials. The excess of recessives is especially noteworthy because defectives of other types frequently show small deficiencies (MANGELSDORF 1926).

The reason for the difference between the percentage of recessives in the 1920 and 1922 crosses apparently lies in the different strains of de_1 which were used as parents. Table 2 shows three successive generations of segregating ears of the original Gold Nugget strain. The proportion of normal to defective seeds in this strain had always approximated a 3:1 ratio and the average percentage of recessives for the entire group is 25.5. It is noted, however, that the two of the ten ears showed an excess of defectives of three or more times the probable error. It so happens that one of these excess ears, 105-9-8-2, was used as the pollen parent in the crosses made in 1922, before the significant deviation from a normal ratio had been noted. The 1920 cross, however, which gave normal segregation in F_2 was made with plants grown from ear 105-9-7 which itself segregated normally. Apparently the excess of recessives in the second generation of the 1922 crosses traces back to some condition which caused an excess in the parental ear.

Table 2

Segregating progenies of three successive generations of inbreeding of a "defective" strain of Gold Nugget.

EAR NO	NORMAL SEEDS	DEFECTIVE SEEDS	DEVIATION	DEVIATION / P.E.	PERCENT DEFECTIVE
105–9	250	73	−8	1.5	22.6
105–9–3	117	49	8	2.2	29.5
105–9–4	159	46	−5	1.2	22.4
105–9–5	146	44	−3	7	23.2
105–9–6	185	49	−9	2.0	20.9
105–9–7	243	75	−5	1.0	23.6
105–9–8	180	78	14	3.0	30.2
105–9–9	213	66	−4	.8	23.7
105–9–8–1	202	61	−5	1.1	23.2
105–9–8–2	134	84	30	7.0	38.5
Total and Average	1829	625	11	.8	25.5

The average percentage of defective seeds, 27.7 percent, in the fourteen ears shown in table 1 represents a significant excess but is not as great a deviation as occurred in the parental ear. When the ears in this group are considered separately, however, it is noted that seven of them show no significant departures from a 3 : 1 ratio; one shows a deficiency and the remaining six an excess greater than three times the probable error. Deviations of three times the error are expected, by chance alone, fairly frequently but in this case half of the ears show marked departures. Clearly the group is not a homogeneous one.

When the six ears which show significant plus deviations are combined, the average for the group is 31.9 percent recessives as compared to 25.1 percent for the non-deviating ears and 20.0 percent for the single ear with a significant minus deviation. In other words this lot of fourteen ears can be divided, on the basis of the deviations, into three distinct groups with *high*, *normal* and *low* defective ratios. A deviation of three times the probable error has been arbitrarily chosen as the dividing point.

If the *high* and *low* defective ears are merely the result of chance deviations, then progenies grown from such ears should give approximately normal ratios in F_3. If, however, these deviations have a genetic basis, distorted ratios might again be expected in F_3.

TABLE 3

Segregating F_3 progenies from "high defective" F_2 ear No. 391.

EAR NO	NORMAL	DEFECTIVE	DEVIATION	DEVIATION / P E	PERCENT DEFECTIVE
Progenies with significant excess					
813	174	85	20	4.3	32.8
822	227	99	18	3.4	30.4
Total	401	184	38	5.4	31.5
Progenies with significant deficiency					
810	108	15	−16	4.9	12.2
811	319	63	−33	5.8	16.5
815	235	56	−17	3.4	19.2
Total	662	134	−65	7.9	16.8
Progenies with no significant deviation					
817	275	83	−7	1.3	23.2
819	130	55	9	2.3	29.7
820	273	89	−2	.4	24.6
Total	678	227	1	.1	25.1
Grand Total	1741	545	−27	1.9	23.8

Third generation progenies of two of the *high* defective ears, 391 and 1520, have been grown and the results are presented in tables 3 and 4.

Twelve progenies were obtained from ear 391 (32.4 percent recessives in F_2), of which eight were segregating for defective seeds. Although the average percentage of recessives for this group is approximately normal, 23.8 percent, it is noted that five of the eight ears deviate from a normal ratio by amounts greater than three times the probable error. Two ears are *high* with an average of 31.5 percent defectives, three are *low* with an average of 16.8 percent recessives and the remaining three are *normal* with an average of 25.1 percent.

In connection with a possible explanation of the distorted ratios in waxy endosperm, presented later, particular attention is called to the difference between the percentage of defectives in the parental F_2 ear (32.4), and the average percentage of defectives in its progenies (23.8). The average of the F_3 progenies approached closely the normal expectation and it is only when the ears are considered separately that irregularities are noted.

Seventeen F_3 progenies were obtained from ear 1520 (33.6 percent recessives in F_2), of which ten were segregating. Four of the segregating ears were *high* with an average of 32.8 percent defectives, one *low*, with 16.8 percent, and five were *normal* with 25.3 percent defectives.

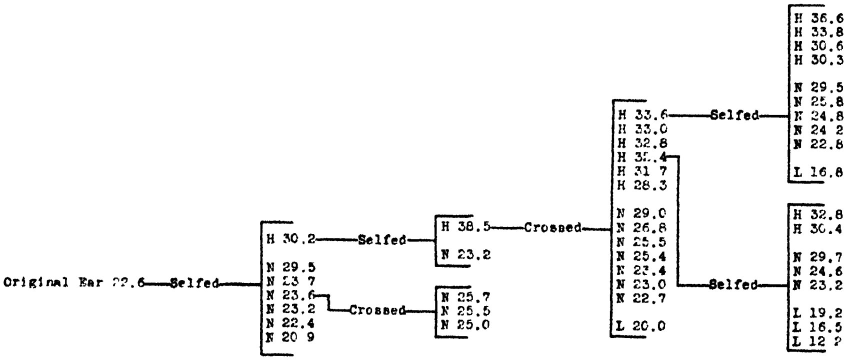


FIGURE 1. Pedigree record of the *de_1* stock showing the inheritance of deviations from normal ratios. The letters H and L represent ears with plus or minus deviations greater than three times the probable error. N represents ears in which the deviation is less than three times the error.

Combining the 18 segregating progenies from these two high defective ears we find that six are *high* with an average of 32.4 percent recessives, four are *low* with 16.8 percent recessives and eight are *normal* with 25.2 percent recessives. The average for the entire F_3 group is 26.4 percent as compared to 33.3 percent for the two parental F_2 ears, a difference of practically seven percent between F_2 and F_3.

Obviously the marked departures from normal ratios in ten of the eighteen F_3 ears cannot be attributed to chance alone. Nor can the results be explained by faulty classification since errors of this sort are usually consistently in one direction. That the marked deviations have a genetic basis is indicated by the fact that they appear in four separate generations as is shown in figure 1.

Apparently the original stock contained a factor or factors which in some way disturbed the normal segregation. The action of this factor first became apparent in the second generation of inbreeding. It has since been transmitted to later generations both in selfed lines of the original stock and in crosses.

TABLE 4

Segregating F_3 progenies from "high defective" F_2 ear No. 1520.

EAR NO.	NORMAL	DEFECTIVE	DEVIATION	DEVIATION / P.E.	PERCENT DEFECTIVE
Progenies with significant excess					
1788	224	99	18	3.5	30.6
1790	156	90	28	6.1	36.6
1792	365	186	48	7.1	33.8
1806	205	89	15	3.0	30.3
Total	950	464	109	10.0	32.8
Progenies with significant deficiency					
1794	188	38	−18	4.1	16.8
Progenies with no significant deviation					
1795	219	70	−2	.4	24.2
1796	167	58	2	.5	25.8
1797	85	28	0	.	24.8
1802	170	71	11	2.4	29.5
1803	227	67	−7	1.4	22.8
Total	868	294	4	.4	25.3
Grand Total	2006	796	96	6.2	28.4

INTERPRETATION OF DEFECTIVE RATIOS

Whenever marked deviations from expected ratios are encountered the possibility of lethal factors at once suggests itself. In maize, however, where a large number of seeds are arranged in a very regular fashion on a single inflorescence, lethal factors, or any sort of differential mortality of the zygotes are readily noted by the occurrence of aborted kernels scattered over the ear. The defective seed in question is, in fact, itself a semi-lethal character.

Lethal factors, moreover, should cause an excess of recessives only when they are linked with the dominant allelomorph of the recessive in question. The marked plus deviations in six of the F_2 ears shown in table 1 could be explained on the basis of lethal factors, only by assuming that each of the five separate seed parents involved in these crosses was heterozygous for a lethal factor linked with the dominant allelomorph of the de_1 gene. The possibility of lethal factors can, therefore, be safely ruled out.

The next hypothesis to be considered is that of differential pollen tube growth. The fact that defectives of other types had frequently given slight deficiencies suggested that these characters, which have such a profound deleterious influence on the sporophyte, might also have some effect in the gametophyte generation, particularly on the rate of pollen tube growth. To test this hypothesis a large number of ears segregating for various types of defectives were arbitrarily divided into upper and lower halves and the proportion of defectives in each half determined separately. If the two types of pollen tubes differ in their rate of growth it might be expected that the greater distance which the tubes were required to travel in reaching the ovules at the base of the ear, would react in favor of the faster growing tubes; that the longer the race the more certain the success of the faster individuals.

TABLE 5

Segregation in upper and lower halves of nine "high defective" ears.

EAR NO	UPPER		LOWER		PERCENTAGE		GAIN IN LOWER HALF
	De	*de*	*De*	*de*	Upper	Lower	
25	75	35	108	55	31.8	33.7	1.9
129	179	83	190	97	31.7	33.8	2.1
173	101	49	99	44	32.7	30.8	−1.9
388	246	91	252	106	27.0	29.6	2.6
391	77	32	50	29	29.4	36.7	7.3
1788	108	45	116	54	29.4	31.8	2.4
1790	77	43	79	47	35.8	37.3	1.5
1792	189	92	176	94	32.7	34.8	2.1
1806	106	40	99	49	27.4	33.1	5.7
Total	1158	510	1169	575	30.6	33.0	2.4

The results of these counts indicated that, with most of the defective types, there was no appreciable difference between the upper and lower halves of the ear in proportion of defectives, and that the defective seed factors do not *per se* reduce the rate of pollen tube growth.

It happened, however, that several of the *high* and *normal* ears of the ***de*$_1$** stock were included in this particular study, without realizing at the time that some of these ears were exceptional. The counts on these ears and several additional ones which have since been examined are shown in tables 5 and 6.

In the *high* ears the average percentage of defectives in the upper halves was 30.6 as compared to 33.0 in the lower halves of the same ears. This difference, though not great, appears to be significant, the odds against its chance occurrence being about 70 to 1 (calculated by Student's formula). The fact that the difference is consistent, all the ears in table 5 with one exception showing a greater proportion of recessives in the lower halves, is regarded as particularly important.

The *normal* ears on the other hand (table 6) show differences in favor of the upper halves as frequently as the lower and the averages of the two halves are very closely alike, 24.6 and 25.5 percent. These results, so far as they go, point to differential rate of pollen tube growth in the *high* ears and equal rate of pollen tube growth in the *normal* ears.

TABLE 6

Segregation in upper and lower halves of seven "normal defective" ears

EAR NO.	UPPER		LOWER		PERCENTAGE		GAIN IN LOWER HALF
	De	*de*	*De*	*de*	UPPER	LOWER	
22	84	27	76	22	24.3	22.4	−1.9
24	75	32	79	31	29.9	28.2	−1.7
42	91	27	93	28	22.9	23.1	.2
126	211	54	217	72	20.4	24.9	4.5
128	142	52	123	38	26.8	23.6	−3.2
389	235	75	189	80	24.2	29.7	5.5
393	160	59	124	38	26.9	23.5	−3.4
Total	998	326	901	309	24.6	25.5	9

Apparently the differential rate of growth is not due to the action of the de_1 factor itself. If pollen tubes carrying this gene were regularly faster or slower in rate of growth, the deviations should regularly occur in the same direction in *all* of the ears. This is not the case. Some ears have marked minus deviations, some marked plus deviations and the remainder segregate normally.

These peculiar results may, however, be explained by assuming that the chromosome pair which carries the De_1 and de_1 factors also bears a gene which influences the rate of pollen tube growth. Since this hypothetical factor has its expression in the gametophyte generation we may give it the factor symbol *Ga*. This factor speeds up the rate of growth in the pollen tubes which carry it and, in the original stock, was linked with de_1, thus enabling a larger proportion of de_1 gametes to accomplish fertilization than would occur by chance alone.

On this assumption the *high* defective plant which served as pollen parent for the 1922 crosses was of the genetic composition *Gade*$_1$, *gaDe*$_1$. Such a plant would produce four kinds of gametes, *Gade*, *gaDe*, *gade*, and *GaDe*. The seed parents of these crosses contributed only one kind of gamete *gaDe*. Thus four distinct classes of F$_1$ plants would be produced as follows:

1. *gaDe GaDe*
2. *gaDe gaDe*
3. *gaDe Gade*
4. *gaDe gade*

These four types would not be expected to occur in equal numbers because their proportion depends upon the amount of crossing over between *Ga* and *de* in the original stock.

Classes 1 and 2 are homozygous for the dominant *De*$_1$ factor and need not be considered further since they do not segregate for defective seeds. Plants in class 4 give segregating ears but the ratios should be normal because the plants are homozygous for *ga* and no differential pollen tube growth can occur. Plants of class 3, however, should, when selfed, give only *high* defective ears because both factors are in the heterozygous condition and the *Ga* factor which speeds up the rate of pollen tube growth is linked with the *de*$_1$ factor which causes defective seeds, thus enabling a larger proportion of the *de*$_1$ gametes to accomplish fertilization than would occur by chance alone.

A glance at table 1 shows that this is exactly the situation in the fourteen F$_2$ ears. Seven *normal* and six *high* ears resulted from these crosses. In addition, however, there is one *low* ear. *Low* ears are not expected from these particular crosses and it is not certain whether ear 392 with 20.0 percent recessives is an exception to theory or merely a chance variation from a *normal* ratio.

It is obvious that in dividing the ears into *high*, *low* and *ncrmal* on the basis of the size of their deviations, some ears which have plus or minus deviations due to chance alone and not to genetic factors, will be included in the two extreme groups. Also some ears which are genetically *high* or *low* will be classed as *normal* because the number of seeds counted is not sufficient to establish the significance of the deviations.

What should happen in F$_3$ when the F$_2$ seeds from a *high* defective ear are grown? The *high* ear, we assume, was borne on a plant of the composition *Gade gaDe*. Such a plant would form four kinds of gametes in the pollen as well as in the ovules and self-pollination should give ten classes of seeds. The genetic constitution of these ten classes and their theoretical behavior when grown and self-pollinated is shown below:

GENOTYPE	BEHAVIOR
1. *GaDe GaDe*	Does not segregate
2. *GaDe gaDe*	Does not segregate
3. *gaDe gaDe*	Does not segregate
4. *GaDe Gade*	Segregates; 3 : 1 ratio
5. *gaDe gade*	Segregates; 3 : 1 ratio
6. *Gade gaDe*	Segregates; *high* defective ratio
7. *GaDe gade*	Segregates; *low* defective ratio.
8. *Gade Gade*	Defective seed
9. *Gade gade*	Defective seed
10. *gade gade*	Defective seed

Genotypes 1, 2, and 3 are homozygous for De_1 and do not segregate. Genotypes 8, 9, 10 are defective seeds and will not grow. Genotypes 4 and 5 segregate but both give normal ratios because the plants are homozygous for *Ga* or *ga* and differential pollen tube growth cannot occur. Plants in genotype 6 are heterozygous for both factors and, since *Ga* and de_1 are linked, should give *high* defective ears. Genotype 7 represents a new combination in which the linkage relations have been reversed. Here the *Ga* factor is linked with the dominant allelomorph and instead of an excess of defectives we should expect a deficiency.

Briefly summarized, then, the seeds from a *high* F_2 ear should, when grown and selfed, give *high*, *low*, and *normal* F_3 progenies. The 18 segregating ears in tables 3 and 4 meet these requirements very satisfactorily. Six are *high*, eight are *normal* and four are *low*. The ratio in which these three types of segregating progenies appear depends not only on the amount of crossing over between *Ga* and de_1 but on the ratio in which the *Ga* and *ga* gametes are presented at fertilization.

It should be emphasized that the validity of this interpretation does not necessarily depend upon the assumption of differential pollen tube growth. Any factor which affects the proportion in which the gametes are presented at fertilization would act in the same manner although its effect might be produced in a number of ways, as for example, by causing differences in the duration of viability or resistance to unfavorable moisture and temperature extremes.

The important fact is that this stock apparently *does* carry a factor which disturbs normal segregation and that this factor is transmitted from generation to generation as is any other Mendelian character. *How* the factor produces its disturbances is another question and one which seems to be partially answered by the data which point to differential pollen tube growth.

DISTORTED RATIOS IN SUGARY ENDOSPERM

The hypothesis of an accessory factor causing differential rate of pollen tube growth has been adopted because the situation is almost identical to that encountered when sugary endosperm is crossed with certain small seeded corneous varieties of the *Zea maize everta* type, known as Squirrel Tooth or Rice Pop.

Although sugary is usually inherited as a simple recessive and gives ratios closely approximating expectation, the Rice Pop × sugary crosses are exceptional in that they always produce a marked deficiency of sugary seeds in the F_2 endosperm generation. This deficiency was first noted by CORRENS (1902) who reported 16 percent sugary seeds in the second generation. EAST and HAYES (1911) also noted the same peculiarity and suggested that the disturbance might be due to faulty classification or to unknown characters contributed by the parents. JONES (1924) and EMERSON (1925) have both made detailed studies of this cross and both have come to the conclusion that the aberrant ratios may be attributed to differential pollen tube growth. EMERSON, however, suggested that the differential rate of growth is not due to the *Su* gene itself but to an accessory factor on the same chromosome. The details of EMERSON's interpretation of this cross have not yet been published and it is not known whether his explanation agrees with the one given here.

The results of the genetic studies of the sugary × pop cross at the CONNECTICUT STATION are briefly summarized in the following paragraphs.

An inbred strain of pointed pop corn was crossed with a first generation hybrid of two inbred strains of sweet corn. One of the sweet strains was a small yellow-seeded variety known as Golden Bantam, the other a large white-seeded variety known as Evergreen. This material was used because it had shown a high degree of selective action in pollen mixtures where pollen from two different types of plants, acting in competition, fertilized more of the ovules of its own type than of the diverse types as previously reported by JONES. (1920, 1922).

In a total of 3681 seeds from eight self-pollinated F_1 plants of this cross shown in table 7, there are only 16.2 percent of sugary seeds. This represents a departure from the normal ratio of 18.3 times the probable error. The percentages of recessives in the individual ears range from 10.5 to 21.4 and the deficiency is greater than three times the probable error in all but one of the ears. In this ear the deviation is 2.6 times the probable error.

F_1 plants of similar cross, though of somewhat different ancestry were backcrossed both ways with the recessive parent. In the backcross of the heterozygote with pollen from the recessive parent (*Susu* × *susu*), 1374 starchy and 1397 sugary seeds were produced. In the reciprocal backcross (*susu* × *Susu*) 885 starchy and 939 sugary seeds occurred. The deviation from a 1 : 1 ratio is not significant in either case. In this respect the results differ from observations with waxy corn which are discussed later. In all this material the segregation of smooth, corneous seeds and wrinkled, glassy seeds was very clear-cut. Occasionally a recessive seed was found that was not deeply wrinkled and at first glance might be classed as a dominant. However, the opportunity for faulty classification was just as great in the F_1 plants backcrossed with the recessive as in the F_1 plants self-pollinated. In the one case a marked deviation was found; in the other, not.

TABLE 7

F_2 progenies of a cross between sugary endosperm and Squirrel Tooth Pop.

EAR NO	STARCHY	SUGARY	DEVIATION	DEVIATION / P E	PERCENT SUGARY
(*Su*×*su*) −1	366	43	−59	10.0	10.5
2	346	94	−16	2.6	21.4
3	376	65	−45	7.3	14.7
(*su*×*Su*) −1	322	56	−39	6.9	14.8
2	378	77	−37	5.9	16.9
3	421	98	−32	4.8	18.9
4	391	47	−63	10.3	10.7
5	485	116	−34	4.8	19.3
Total	3085	596	−324	18.3	16.2

The next step was to backcross the F_1 plants with the dominant parent (*Susu* × *SuSu* and *SuSu* × *Susu*). Since the result of fertilization by a recessive or dominant carrying gamete would be obscured, because all the seeds would have the dominant endosperm condition, it was necessary to grow the plants and determine which were segregating and which were not. This was done by planting the backcrosses, made both ways, and allowing all of the plants to interpollinate naturally. Any plants that resulted from *Su* × *Su* fertilization would show no recessives, while most of the plants of the composition *Su* × *su* would be expected to show some recessive seeds. With no selective action one-half of the plants would be segregating so that one-fourth of the pollen in the field would be carrying the recessive factor. Segregating plants would be expected to have half of their ovules with the recessive factor so that segregating ears should have, on the average, one-eighth of their seeds recessive. Selective pollination working against the recessive gametes would reduce this proportion. Only one ear was taken from each plant and most of these contained several hundred kernels. All ears which had any sugary seeds were classed as segregating. The number of sugary seeds varied on different ears but the number of plants which had no recessive seeds and for that reason would be wrongly classified was probably less than seven percent and was not likely to be enough to disturb the results seriously in view of the wide departure from the normal 1 : 1 ratio obtained. The backcross of pollen from the homozygous dominant parent on the heterozygous F_1 plants (*Susu* × *SuSu*) gave 207 segregating and 213 non-segregating individuals. These numbers differ from a 1 : 1 ratio less than the probable error. But the pollen from the heterozygous F_1 plants backcrossed on the homozygous dominant parent (*SuSu* × *Susu*) gave 88 segregating and 353 non-segregating individuals, a deviation from a 1 : 1 ratio of 18.7 times the probable error.

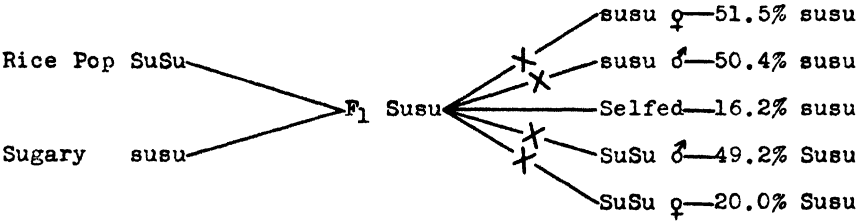


FIGURE 2. Diagram showing the behavior of the Rice Pop×Sugary cross when selfed and backcrossed reciprocally with both parents.

To sum up, the F_1 plants self-fertilized showed a selective action such that more of the gametes carrying the dominant factor united than would be the case in random mating. The heterozygous F_1 plants backcrossed with the recessive parent showed no selective action either way the pollinations were made. Backcrossed with the dominant parent there was no selective action when the pollen was alike but the pollen from the heterozygous F_1 plants showed a markedly greater pollinating ability on the part of the gametes carrying the dominant factor. These results are shown graphically in figure 2.

This series of facts shows that the selective action is not due to differences in the functioning of the pollen alone, although the differential action is exhibited only when the pollen is diverse, otherwise unequal numbers would be obtained in the backcross on the recessive parent as well as on the dominant.

As a result of these experiments JONES (1924) concluded that: "There is apparently an interaction between the pollen tube and the tissues in which it grows, such that pollen carrying the dominant factor is better able to accomplish fertilization than the pollen carrying its recessive allelomorph, only in a sporophyte which also has the dominant factor either in the haploid or diploid state."

At that time it was thought that the *Su* factor itself was responsible for the speeding up of the pollen tubes in certain combinations. It now seems more probable that the results are due to linkage of the *Su* factor with a gametophyte factor (*Ga*). If the linkage relations between *Su* and *Ga* were reversed the results would be entirely different but the above statement as applied to the *Ga* factor would still hold.

The factor symbol for the gametophyte factor has been written in capital letters as though it were a true dominant. It may be questioned whether this is legitimate since the *Ga* factor has its expression in the gametophyte where it necessarily occurs in a haploid condition and is not required to compete with its allelomorph. The *Ga* pollen tubes are given an advantage, however, only when the sporophytic tissue in which they grow also has this factor, and the fact that in the sporophyte it is apparently as effective when heterozygous as when homozygous indicates that it is a dominant factor.

On the basis of a gametophyte factor linked with the *Su* factor, the combinations here discussed may be given the following factorial representation:

TYPE OR COMBINATION	COMPOSITION OF PLANT	MALE GAMETES			
Rice Pop parent	*SuSuGaGa*	*SuGa*			
Sugary parent	*susugaga*	*suga*			
F_1 selfed	*SusuGaga*	*SuGa*	*Suga*	*suGa*	*suga*
$F_1 \times$ sugary parent	*SusuGaga*	*suga*			
Sugary parent $\times F_1$	*susugaga*	*SuGa*	*Suga*	*suGa*	*suga*
$F_1 \times$ starchy parent	*SusuGaga*	*SuGa*			
Starchy parent $\times F_1$	*SuSuGaGa*	*SuGa*	*Suga*	*suGa*	*suga*

With this factorial composition in mind the results reported in the preceding paragraphs are readily understood on the assumption that the *Ga* factor is effective in speeding up pollen tube growth only in a sporophyte which has the *Ga* factor in the homozygous or heterozygous state.

The F_1 selfed shows a selective action because *Ga* and *ga* pollen tubes compete with each other in sporophytic tissue which has the *Ga* factor in the heterozygous condition. The *Ga* tubes are given an advantage under these conditions and since *Ga* and *Su* are linked, more *Su* than *su* gametes, reach the micropyle and accomplish fertilization. F_1 plants pollinated by the sugary parent show no selective action because only one type of male gamete is involved. The backcross of the sugary parent by the F_1 involves four types of male gametes but no selective action is shown because the sporophyte in which the pollen tubes must grow lacks the dominant *Ga* factor. F_1 backcrossed by the starchy parent shows no selective action because only one kind of male gamete is present. But the backcross of the starchy parent by the F_1 again shows a very marked selective effect because *Ga* and *ga* gametes are competing in sporophytic tissue which has the dominant *Ga* factor in the homozygous state.

The F_2 progenies which show such a marked deficiency of sugary seeds, should, when selfed, produce the same three types of segregating progenies as did the *high* defective ears when selfed. Here of course the proportion of the three types would be reversed and (1) the majority should be deficient in sugary seeds, (2) some should segregate normally, and (3) a few should give an excess. EMERSON has grown such progenies and has obtained exactly these results, his *low* ears giving 15 percent recessives, his *high* ears 35 percent. The writers have also grown a few F_3 progenies from this cross and have obtained the expected types, although in the case of the *high* sugary ears the number of seeds counted was not great enough to prove that the deviations were statistically significant.

LINKAGE BETWEEN *Su* AND de_1

The question at once arises whether the deviations found in the de_1 stock are caused by the same accessory factor, always present in Rice Pop, which causes such marked disturbances in the starchy: sugary ratio. To determine this point crosses of *high* defective × pop, *low* defective × pop, *high* defective × sugary, and *low* defective × sugary have been made and the F_2 endosperm generation of these crosses will be available in another season. In the meantime, indirect evidence that the accessory factor in both stocks is probably the same is available in a cross of *normal* defective × sugary, which had been made for another purpose. Four F_2 progenies of this cross are shown in table 8.

It is noted that the two parental classes are in excess, while the two new combinations show a deficiency. When parental classes are combined and compared to the new combinations in the form of a 10 : 6 ratio, the deviation is found to be 4.9 times the probable error. Such a deviation should occur by chance alone only once in 1052 trials but can readily be explained by assuming linkage (repulsion phase) between *su* and de_1. The percentage of crossing over as determined from the normal seeds is 38.5; determined from the defective class it is 39 percent.

RELATIVE EFFECTIVENESS OF *Ga* AND *ga* GAMETES

Knowing that the de_1 and *su* factors are linked and that both are affected to a marked degree by an accessory factor located on the same chromosome, it is almost certain that the same accessory factor is acting in both stocks. On such an assumption it is possible to estimate approximately the position on the chromosome of the *Ga* factor in relation to the *su* and de_1 genes and at the same time to determine roughly the ratio of *Ga* to *ga* gametes which accomplish fertilization.

The presence of gametophyte factors which disturb the equality in which two kinds of gametes are presented at fertilization, brings up entirely new problems in Mendelian segregation. The amount of crossing over between a gametophyte factor and a sporophyte factor may range from 0 to 50 percent. At the same time the ratio in which the *Ga* and *ga* gametes occur at fertilization may range from 1 : 1 to 1 : 0.

TABLE 8

Segregation in F_2 of a cross between sugary endosperm and defective seed.

EAR NO	NORMAL SEEDS		DEFECTIVE SEEDS	
	Starchy	Sugary	Starchy	Sugary
947	71	36	42	10
951	213	89	75	27
953	219	81	85	18
956	98	32	45	9
Total	601	238	247	64
Ex 9:3:3:1	647	216	216	72
Deviation	−46	22	31	−8

Grouping into a 10 : 6 ratio
Found 665 485
Ex. 10 : 6 *719* *431*
Deviation 54±11.1
Dev./P. E. 4.9

The amount of crossing over and the relative effectiveness of the accessory factor are, then, the two variables which determine the size of the deviations from normal ratios. Table 9 shows the theoretical percentage of recessives which should occur with different combinations of these two variables, assuming that a gametophyte factor *G* which increases the chances of the gametes, in which it occurs, to accomplish fertilization, is linked with the dominant allelomorph *S* of a recessive sporophyte factor. If the linkage were reversed (*Gs gS*), the deviations would be of the same magnitude but would occur in the opposite direction. The size of the deviations produced by different combinations of the two variables is also illustrated in figure 3.

The average percentage of defective seeds in the *high* ears of tables 3 and 4 is 32.4, a deviation of 7.4 percent from normal. The *low* ears from the same families average 16.8 percent recessives, a deviation from normal of 8.2 percent. The average deviation for both groups is 7.8 percent. In the *low* sugary ears of table 7 the average deviation from a normal ratio is 8.8 percent.

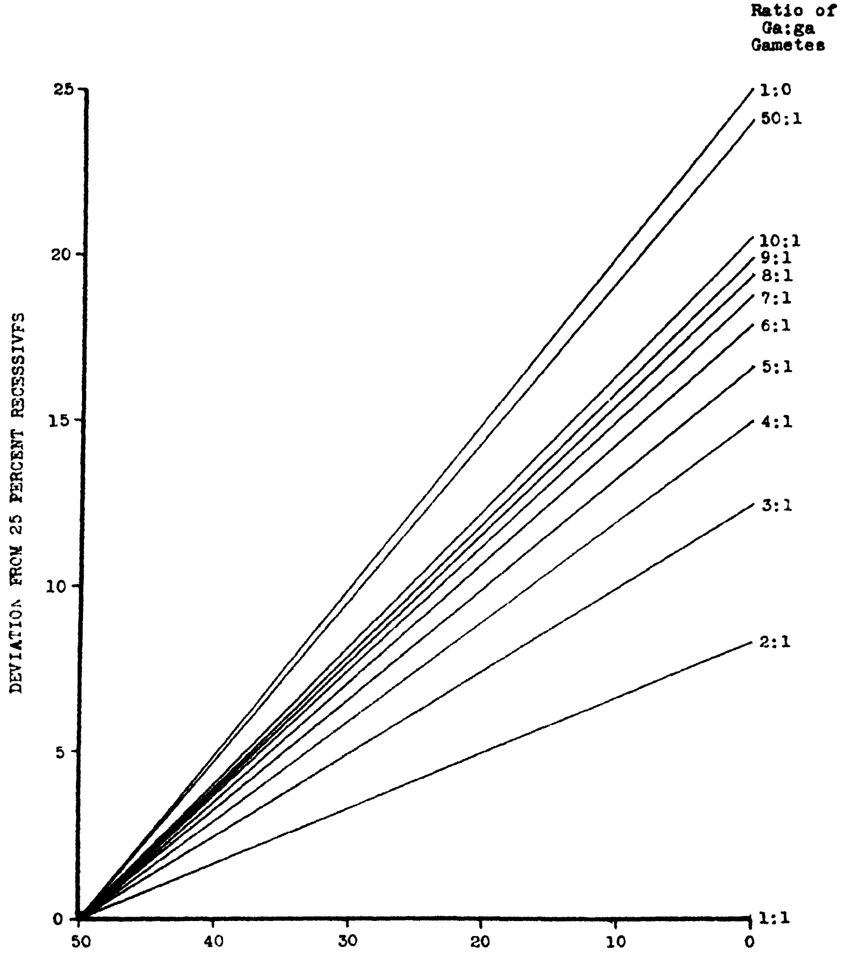


FIGURE 3. Diagrams showing the theoretical deviations from 25 percent recessives resulting from various combinations of crossing over and relative effectiveness of two kinds of gametes in accomplishing fertilization.

A deviation of 7.8 percent in the defective seed ratios and 8.8 percent in the sugary ratios might be caused by any number of combinations of crossing over and differential effectiveness of the *Ga* and *ga* gametes. This is illustrated by the curves in figure 3. Thus if the value of one of these influences were known it would be a fairly simple matter to determine the value of the other. Unfortunately this is not the case.

It is known, however, that the crossing over between *Su* and *de*$_1$ is approximately 39 percent. An accessory factor could affect the two almost equally, only if it were approximately the same distance from both. This means that its locus must lie between the *Su* and *de*$_1$ loci.

TABLE 9

Percentages of recessives (ss) expected when a factor G which stimulates the rate of pollen tube growth is linked with the dominant allelomorph S of a recessive character s.

RATIO IN WHICH GAMETES ARE PRESENTED	PERCENTAGE OF CROSSING OVER BETWEEN *G* AND *S*									
	0	5	10	15	20	25	30	35	40	45
1 : 1	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
2 : 1	16.65	17.50	18.33	19.17	20.00	20.83	21.67	22.50	23.33	24.17
3 : 1	12.50	13.75	15.00	16.25	17.50	18.75	20.00	21.25	22.50	23.75
4 : 1	10.00	11.50	13.00	14.50	16.00	17.50	19.00	20.50	22.00	23.50
5 : 1	8.33	10.00	11.67	13.33	15.00	16.67	18.33	20.00	21.67	23.33
6 : 1	7.14	8.93	10.72	12.50	14.29	16.07	17.86	19.64	21.43	23.22
7 : 1	6.25	8.13	10.00	11.88	13.75	15.63	17.50	19.37	21.25	23.12
8 : 1	5.56	7.50	9.45	11.39	13.33	15.28	17.22	19.17	21.11	23.06
9 : 1	5.00	7.00	9.00	11.00	13.00	15.00	17.00	19.00	21.00	23.00
10 : 1	4.55	6.59	8.64	10.68	12.73	14.77	16.82	18.86	20.90	22.95
20 : 1	2.38	4.64	6.91	9.17	11.43	13.69	15.95	18.21	20.48	22.74
30 : 1	1.61	3.95	6.29	8.63	10.97	13.31	15.64	17.98	20.32	22.66
40 : 1	1.22	3.60	5.98	8.35	10.73	13.11	15.49	17.87	20.24	22.62
50 : 1	.98	3.38	5.78	8.19	10.59	12.99	15.39	17.79	20.20	22.60
1 : 0	.00	2.50	5.00	7.50	10.00	12.50	15.00	17.50	20.00	22.50

TABLE 10

Crossing over values and map distances indicated by ordinates erected at different points on abscissa in figure 4.

POINT ON ABSCISSA	PERCENTAGE CROSSING OVER		MAP DISTANCE		TOTAL MAP DISTANCE
	Ga – *Su*	*Ga* – *de*$_1$	*Ga* – *Su*	*Ga* – *de*$_1$	
3.7	19.3	22.7	20.9	25.0	45.9
3.8	19.8	23.1	21.5	25.5	47.0
3.9	20.3	23.5	22.1	26.0	48.1
4.0	20.8	23.9	22.7	26.5	49.2
4.1	21.2	24.3	23.2	27.0	50.2
4.2	21.5	24.7	23.6	27.5	51.1
4.3	21.8	25.0	23.9	27.9	51.8
4.4	22.1	25.3	24.2	28.3	52.5

The map distance between *Su* and *de*$_1$ is 50 units as determined from HALDANE'S table (HALDANE 1919). These tables are, of course, based on the results from the sex chromosome in Drosophila but since they have been found to apply equally well to the other chromosomes of Drosophila and to the linkage groups in Primula, it is probable that they hold for the linkage groups in maize.

The problem in this case is to determine at what point on the abscissa in figure 4 an ordinate can be erected which crosses the two curves at points, whose values converted into map distances and combined, equal 50 units. Table 10 shows the results of erecting ordinates at a number of points along the abscissa.

It is noted that an ordinate erected at 4.1 crosses the two curves at points whose values best fit the facts. In other words, if 4.1 times as many *Ga* as *ga* gametes accomplish fertilization, then the crossing over between *Ga* and *Su* is 21.2 percent and between *Ga* and de_1, 24.3 percent. The map distance between *Ga* and *Su* would then be 23.2 units and between *Ga* and de_1, 27.0 units. These two values combined equal 50.2 units which is approximately the map distance between *Su* and de_1.

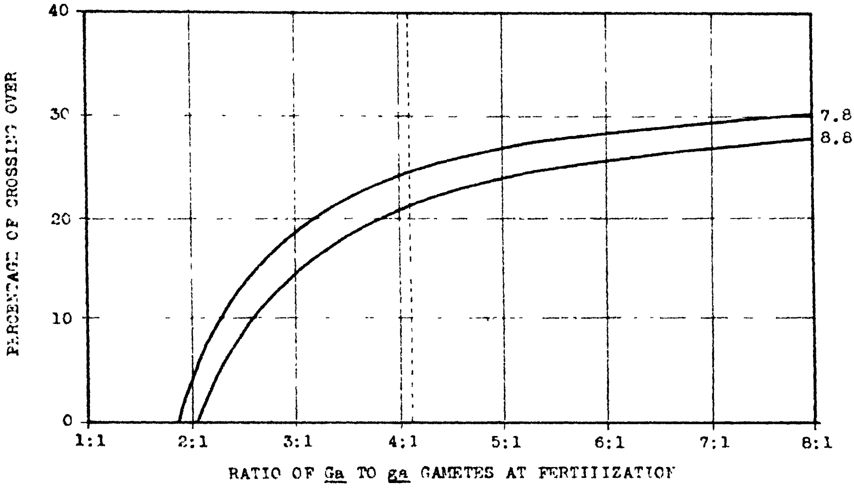


FIGURE 4. The various combinations of crossing over and relative effectiveness of two kinds of gametes which might cause deviations of 7.8 and 8.8 percent from the expected 25 percent recessives. An ordinate erected at 4.1 crosses the two curves at points whose values converted into map distances and added, equal the map distance between de_1 and *Su*.

It is realized, of course, that it is impossible to arrive at more than a crude approximation by this method. It is difficult to determine the true value of either of these influences until one of them is more accurately known. Furthermore, the assumption has been made that the relative proportion of *Ga* and *ga* gametes which accomplish fertilization is always the same. This is probably not strictly true. The data already given, and further data from mixed pollinations presented later, indicate that the selective action is due to differential pollen tube growth. The ratio of the two classes of gametes at fertilization would, therefore, depend to some extent on length of styles, weather conditions, and perhaps many other factors. In the long run these various influences would be expected to balance each other and over a period of years the ratio of the two types of gametes would be relatively constant. In comparing two series of observations, however, a variation in the relative effectiveness of the *Ga* and *ga* gametes must be kept in mind.

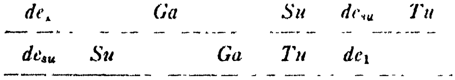
PREDICTING THE SIZE OF THE DEVIATIONS

If these approximations have any value it should now be possible to predict the deviations which will occur when other characters on the sugary chromosome are crossed with Rice Pop, providing that the amount of crossing over between sugary and the new character is known. A recent paper by WENTZ (1925) includes data which answer this purpose admirably. WENTZ found that a defective seed type de_{su} is linked with sugary with 3.2 percent crossing over. Since the crossing over between the *Ga* and *Su* loci is approximately 21.2 percent, then the crossing over between *Ga* and de_{su} should be about 23.8 or 18.6 percent depending on the direction of the de_{su} gene from the sugary locus. With the *Ga* and *ga* gametes occurring at fertilization in the ratio of 4 to 1, a cross between Rice Pop (*GaDe GaDe*) and WENTZ's defective (*gaDe gade*) should produce either 17.1 or 15.6 percent recessives on the segregating ears. Fortunately, WENTZ has crossed his defective seed strain with a stock tracing back to Rice Pop ancestry and apparently heterozygous for the accessory factor of the Pop parent. Eight segregating ears in the F_2 endosperm generation of this cross gave an average of 19.8 percent defective seeds as is shown in table 1 of WENTZ's paper. This value differs from the value predicted above by 2.7 percent.

Through the kindness of Professor WENTZ the detailed data on the individual ears of this cross, which were not included in his published paper, were made available to us. These data showed that three of the eight ears gave approximately normal ratios averaging 24.7 percent recessives. The other five ears, however, all deviated from a normal ratio by significant amounts and when combined gave a total of 1475 normal and 309 defective seeds, an average of 17.3 percent recessives.

The agreement of this value with the 17.1 percent recessives predicted by the above calculations is surprisingly good and is either a remarkable coincidence or an excellent bit of evidence in substantiation of our hypothesis.

The position of the factors on the sugary chromosome is, then, approximately as shown in one or the other of the two following diagrams.



Which of these arrangements is the correct one will be determined when the character *tunicate ear* is crossed with Rice Pop. The crossing over between *su* and *Tu* is approximately 28.6 percent (EYSTER 1921). In the first arrangement *Tu* is so far from *Ga* that its segregation would be affected only very slightly by the differential pollen tube growth. In the second arrangement *Tu* is fairly close to *Ga* and if this is the correct one, marked deviations should occur in the tunicate ratios when this character is crossed with Rice Pop or other stocks carrying the *Ga* factor.

GAMETOPHYTE FACTORS AND MIXED POLLINATIONS

The presence in Rice Pop of a gametophyte factor which influences the rate of pollen tube growth in sporophytic tissue of the same genetic constitution, probably accounts to a large extent for the results obtained in mixed pollination experiments. (JONES 1920, 1922). In these tests where a mixture of the white pop type and a yellow seeded sugary type was applied to the silks of both, there was a marked preference of the plants for their own kind of pollen. The results of five such mixed pollinations are shown in table 11.

TABLE 11

The amount of selective action shown by maize in five pollen mixtures.

POLLEN MIXTURE NO	NUMBER OF SEEDS				TOTAL NO. OF SEEDS	DEVIATION FROM PERFECT PROPORTION IN PERCENT
	A×A	A×B	B×A	B×B		
1	811	11	381	2006	3209	41.35
2	4222	27	466	1404	6119	37.22
3	1568	2	319	224	2113	20.56
4	1930	29	73	309	2341	39.71
5	4084	6	963	290	5343	11.50

If there were no differences in pollinating ability, the proportion should be a perfect one within the limits of random sampling. The white smooth-seeded plants are designated as A in the tables and the plants with yellow, sugary seeds are listed under the heading of B. Without selective action the ratio of crossed to selfed seeds should be the same on ears of both types, regardless of the amount or viability of each kind of pollen. The last column gives the deviations from the closest calculated perfect proportion based upon the percentage of cross-fertilized and self-fertilized seeds on each type of plant. The maximum deviation of 50 percent would indicate complete functioning of each kind of pollen on its own flowers to the exclusion of the foreign type. The deviations actually range from 14 to 41 in favor of self-fertilization. In some tests the deviations have been even higher but only the above five groups are given here because these were also used in determining differences in pollen tube growth.

That the selective action is due, in part at least, to differences in pollen tube growth, is indicated by the data in table 12. The ears were arbitrarily divided into top and bottom halves before shelling. The distance that the pollen tubes had to travel differed considerably in the case of seeds produced at the tip of the spike as compared with those at the base. If the plant's own pollen tubes grew more rapidly than the foreign tubes, we would expect fewer cross-fertilized seeds at the base than at the tip of the spike.

TABLE 12

The ratio of crossed seeds in upper and lower halves of ears resulting from mixed pollinations.

POLLEN MIXTURE NUMBER	PLANT TYPE	TOTAL NO OF SEEDS	NO OF CROSSED SEEDS PER 100		RATIO OF CROSSED SEEDS TOP : BOTTOM
			TOP	BOTTOM	
1	Pop	822	1.99	.54	3.7 : 1
1	Sugary	2387	17.23	14.64	1.1 : 1
2	Pop	4249	1.22	.05	24.4 : 1
2	Sugary	1870	26.66	23.01	1.2 : 1
3	Pop	1570	.25	.00	.25 : 0
3	Sugary	543	60.07	57.14	1.1 : 1
4	Pop	1959	2.54	.32	7.9 : 1
4	Sugary	382	24.73	13.78	1.8 : 1
5	Pop	4090	.10	.20	.5 : 1
5	Sugary	1253	78.43	75.35	1.1 : 1
Total and Average	Pop	12,690	1.22	.22	5.6 : 1
	Sugary	6,435	41.42	36.78	1.1 : 1

The last column in table 12 shows the ratio of crossed seeds in the top halves to those in the bottom halves. In all but one mixture there was a greater proportion of crossed seeds in the top halves of the ears and in this one exception there were only six crossed seeds in a total of four thousand. It is also noted that the excess of crossed seeds in the upper part of the inflorescence is much greater on ears of the pop type than those of the sugary type. In the former there were 5.6 times as many crossed seeds in the top halves while in the sugary ears there were only 1.1 times as many crossed individuals in the upper part of the inflorescences. The marked selective action found in these mixtures is apparently due to a condition in the pop parent which permits the plant's own pollen tubes to grow more rapidly in its own styles. That this condition is brought about by a gametophyte factor on the sugary chromosome has already been indicated.

It will be noted that the data also indicate a slight preference of the sugary plants for their own kind of pollen. Probably there are many genetic factors which affect pollen tube growth and any of these which cause increased growth when the sporophytic tissue is of the same genetic constitution, will give the plants' own pollen an advantage over foreign pollen. Almost all pollen mixtures show a slight selective action in favor of the plants' own pollen but only where one of the types possesses a factor which causes a marked increase in the rate of pollen tube growth, such as is found in Rice Pop, is the selective action so decided.

DISTORTED RATIOS IN WAXY ENDOSPERM

The deficiency of waxy seeds originally noted by COLLINS and KEMPTON (1911) in the F_2 endosperm generation of hybrids between waxy and other varieties of maize can also be explained, at least partially, by the action of one or more gametophyte factors linked with the *Wx wx* pair.

New interest in the inheritance of waxy has recently been aroused by the discovery that the carbohydrate reserve in the waxy endosperm is of a different chemical nature than that of other varieties of maize, staining red with iodine, while other types of endosperm stain a deep blue (WEATHERWAX 1922), and that this difference in reaction to staining is also apparent in the pollen grains (BRINK and MACGILLIVRAY 1924, DEMEREC 1924, LONGLEY 1924) and in the embryo sac. (BRINK 1925, KIESSELBACH and PETERSEN 1926)

KIESSELBACH and PETERSEN (1926) have summarized all the available data on the inheritance of waxy endosperm, including considerable new data of their own. When all the results are combined there is a deficiency of 1.1 percent from the expected 25 percent when the heterozygote is selfed and .7 percent from the expected 50 percent when segregating pollen from heterozygous plants is applied to the pure recessives. These writers conclude that "the evidence at hand seems insufficient to definitely establish the causes or significance of the deviations", although the deviation in the first case is 14.8 times the probable error and in the second 5.4 times the error.

The difficulty of interpreting the waxy situation is not due to a deficiency of data but to the fact that data, which are not strictly comparable, have been combined. Different investigators have used different stocks in crossing with waxy, and the waxy stock itself after repeated crossings and extractions, since it was first brought from China, is probably far from homogeneous. If accessory factors are involved in the inheritance of waxy it will be readily seen that the procedure of combining data from a wide variety of sources and especially that of combining different generations, is not a sound one. Merely adding a few thousand individuals to the third of a million already counted will contribute very little toward solving the cause of the deviations.

The fallacy of combining the data from separate generations is well illustrated by the defective seed ratios in table 3. An F_2 ear with 32.4 percent recessives produced F_3 progenies with an average of 23.8 percent recessives. At first glance it appeared that the deviation in F_2 was not inherited. Closer examination, however, shows that although the average percentage of defective seeds in F_3 is almost normal, the individual progenies show marked deviations, some in one direction, some in another

ANALYSIS OF KEMPTON'S DATA

That a somewhat similar situation exists in the waxy stocks is shown by data from KEMPTON (1919). KEMPTON'S F_2 progenies gave an average of 23.7 percent waxy; his F_3 progenies, an average of 24.6 percent waxy. The difference between these two generations is 4.3 times the probable error and would be expected as the result of chance only once in about 267 trials.

The difference between the second and third generations cannot be explained by consistent errors in classification or to regular differences in viability, rate of pollen tube growth, or resistance to unfavorable influences inherent to the waxy factor *itself*, since all of these influences would produce as great a disturbance in one generation as in another. The situation, however, is exactly what would be expected if an accessory factor linked with the waxy gene is involved.

Further evidence of the action of an accessory factor influencing the waxy ratio is found in the data in table 1 of KEMPTON'S paper (1919). In this table are given all the results of selfing and intercrossing F_1 plants. Ordinarily, of course, these two types of pollination should give identical results but if an accessory factor is present in part of the plants, differences might well occur. KEMPTON, in intercrossing a pair of F_1 plants, frequently also self-pollinated one or both members of the pair and the data resulting from selfing are, fortunately, included in the table and can be compared to those obtained from the intercrossing.

A study of the pedigree numbers in this table show that there are 26 intercrosses in which the segregation of the two F_1 plants used in making the cross, has also been determined by selfing. Some of the F_1 plants gave almost normal ratios, others showed a deficiency of waxy seeds. Arbitrarily dividing the selfed plants into *normal* and *low*, using a deviation of twice the probable error as a basis, the intercrosses can be arranged into the following groups; *low* × *low*, *normal* × *low*, *low* × *normal*, and *normal* × *normal*.

Obviously, if the deviations in the selfed plants are merely due to chance, then grouping the intercrosses on the basis of the segregation in the selfed parental plants can have no effect on the average percentage of waxy in each group. If, however, the deviations are due to some condition inherent in the plants, it might be expected that *low* × *low* intercrosses would give different results than *normal* × *normal* pollinations. The results of grouping the intercrosses according to the behavior of both members of the parental pair are shown in table 13.

It is noted that the *low* by *normal* and *normal* by *normal* intercrosses are very closely alike, giving 25.1 and 25.4 percent of waxy seeds respectively. The *normal* by *low* and *low* by *low* intercrosses also resemble each other giving 22.9 and 22.6 percent of waxy seeds respectively. In other words, the groups of intercrosses in which pollen from *normal* plants was used gave *normal* ratios, the average for the two groups being 25.3 percent recessives, a very close approximation to the theoretical ratio. When pollen from *low* waxy plants was used, however, the intercrosses gave *low* waxy ratios, the average for the two groups being 22.7 percent waxy, a deviation from the theoretical ratio of 5.8 times the probable error.

It should be mentioned that in addition to the intercrosses shown in table 13 there are two involving plants with *high* waxy ratios. One of these, *normal* × *high*, gave 21.3 percent waxy, the other, *high* × *low*, 28.1 percent waxy. No conclusions regarding the significance or meaning of plus deviations can be drawn from these two ears.

It is apparent that the arbitrary grouping of the F_1 plants into *low* and *normal* on the basis of their deviations has actually divided them into classes which are inherently distinct. Plants giving *low* ratios when selfed give almost exactly the same ratios when their pollen is used on other plants. The remaining plants give ratios closely approximating *normal* whether selfed or intercrossed. In other words, *the random assortment of the male gametes at fertilization is disturbed in about half of the plants, and is relatively normal in the remainder.*

These facts can readily be explained by assuming that one of the parents of KEMPTON'S hybrids was heterozygous for an accessory factor which affected the chances of the two kinds of gametes in accomplishing fertilization. Probably it was the waxy stock which brought in the accessory gene because deviations have been found in almost every case that waxy has been crossed with other stocks.

With the evidence of an accessory factor in mind it should be possible to isolate from such crosses as KEMPTON'S, some heterozygous waxy lines which regularly give normal ratios, some which give a majority of minus deviations and others a majority of plus deviations. We have no doubt that such lines will soon be reported and that crosses with other characters on the waxy chromosome will indicate the approximate location of the accessory factor. BRINK (1925) has, in fact, evidence from one of his crosses which indicates the presence of an accessory factor closely linked with the *I* factor, which is located on the waxy chromosome.

The gametophyte factor in the waxy stock probably disturbs the ratios by causing differential pollen tube growth as does the factor in the sugary and defective seeds stocks. At least this is the simplest explanation and there is some evidence of its correctness from BRINK'S (1925) experiments in which he found that fewer waxy seeds were produced on ears with long styles compared to those in which the styles were cut short.

PECULIAR RATIOS IN COLOR SEGREGATION

It is not improbable that COULTER'S aberrant segregation in a stock heterozygous for the *C* factor (COULTER 1925) is due to the same accessory factor which affects the waxy ratios, since *C* and *wx* are members of the same linkage group.

COULTER'S stock produced some progenies with marked minus deviations of white seeds, some with marked plus deviations, and others with normal ratios. He explains the deviations on the basis of linkage with a zygotic lethal factor and it is true that they can be fairly satisfactorily explained on this basis, although some of his *high* white ratios are higher than would be expected. Zygotic lethals, even with complete linkage, (repulsion phase) should cause a distortion of the ratio only from a 3 : 1 to a 2 : 1. A large proportion of COULTER'S high ears average higher than 33.3 percent recessives and a number of them show 38 to 40 percent of white seeds.

Furthermore, zygotic lethals in maize are usually expressed in the form of defective seeds, and when these are present they can be readily noted and their numbers accurately determined. COULTER asserts that aborted seeds are sometimes found in this stock but apparently not regularly. He writes[1] "Many of my ears have the random distribution of empty pericarps so characteristic of the defectives. On other ears it seems impossible to detect the presence of the abortive grains. Doubtless accessory factors are at play to determine the degree of expression of this character."

From the experience in studying the inheritance of fourteen types of defective seeds, (MANGELSDORF 1926), it may be said that when defective seeds are present at all, their segregation is usually clear-cut and their number can be readily determined. The accessory factor which COULTER postulates is probably not one which so affects the degree of expression of the defectives that they can no longer be distinguished from the normal seeds but rather a gametophyte factor which influences the ratio in which the defective seeds appear, as well as the segregation for color. If this is true it should be possible to isolate from COULTER'S stock, lines which give *high*, *normal* and *low* white progenies but which do not segregate for defective seeds, others which give *high*, *normal*, and *low* defective progenies but do not segregate for color.

HOW GAMETOPHYTE FACTORS OPERATE

If the gametophyte factor on the waxy chromosome is one which affects the rate of pollen tube growth, it differs in two respects from the *Ga* factor on the sugary-defective chromosome. The first difference is that the waxy pollen tube factors reduces the rate of pollen tube growth instead of stimulating it. The second difference is that it operates regardless of the genetic constitution of the sporophytic tissues in which the pollen tubes grow. This is shown by the fact that the *normal* × *low* intercrosses in table 13 gave exactly the same results as the *low* × *low*.

There is, of course, no reason for believing that all gametophyte factors behave in the same way. It is probable that factors operating in the gametophyte generation may have their effect in many different ways, for example; in causing differential pollen tube growth, duration of viability, resistance to drying out, excessive moisture, high and low temperature, etc. Even the factors affecting the rate of pollen tube growth alone may fall into at least four distinct categories, as follows:

1. Factors which reduce the rate of pollen tube growth only when the tubes are growing in sporophytic tissue of the same genetic constitution.
2. Factors which reduce the rate of pollen tube growth regardless of the genetic constitution of the sporophytic tissues.

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[1] In a letter.

3. Factors which stimulate the rate of pollen tube growth only when the tubes are growing in sporophytic tissue of the same genetic constitution.
4. Factors which stimulate pollen tube growth regardless of the genetic constitution of the sporophytic tissues.

TABLE 13

Kempton's F_1 intercrosses grouped on the basis of the segregation in the selfed parental plants.

TYPE OF INTERCROSS	PEDIGREE NUMBERS			PERCENTAGE WAXY		
	♀	♂	INTERCROSS	♀	♂	INTERCROSS
L×L	1534	1743	1535	21.7	20.5	22.7
L×L	1543	1723	1542	20.9	21.0	23.3
L×L	1723	1543	1722	21.0	20.9	23.9
L×L	1743	1534	1742	20.5	21.7	21.3
Average						22.9
N×L	1117	1118	1116	24.2	21.9	26.0
N×L	1515	1726	1518	25.0	21.6	21.5
N×L	1129	1135	1130	24.1	21.5	21.0
N×L	1133	1118	1134	24.6	21.9	16.4
N×L	1137	1135	1138	26.0	21.5	25.1
N×L	1741	1551	1740	22.7	21.2	25.6
Average						22.6
L×N	1111	1132	1110	22.2	23.3	27.5
L×N	1118	1108	1119	21.9	26.4	25.8
L×N	1121	1117	1120	22.5	24.2	22.1
L×N	1551	1741	1550	21.2	22.7	20.6
L×N	1135	1137	1136	21.5	26.0	28.6
L×N	1726	1519	1725	21.6	23.8	24.2
Average						25.1
N×N	1105	1108	1104	26.4	26.4	23.7
N×N	1527	1745	1526	23.3	23.8	26.8
N×N	1549	1749	1548	24.6	26.3	24.0
N×N	1128	1125	1127	25.0	24.0	24.4
N×N	1132	1117	1131	23.3	24.2	22.9
N×N	1721	1515	1720	23.2	25.0	30.7
N×N	1745	1527	1744	23.8	23.3	26.0
N×N	1749	1549	1748	26.3	24.6	25.8
Average						25.4

Gametophyte factors which fall into the second and third of these categories have apparently already been found in maize in the waxy and sugary-defective pollen tube factors. An example of the first group is found in the inheritance of self-sterile classes in Nicotiana (EAST and A. J. MANGELSDORF 1925, 1926) where certain classes are almost completely eliminated presumably because of the slower rate of pollen tube growth.

CARBOHYDRATE METABOLISM AND POLLEN TUBE GROWTH

It may have been noted that all the characters so far reported *su, wx*, de_1 and de_{su} which appear to be associated with differences in pollen tube growth, are endosperm characters which are also associated with differences in the nature or amount of carbohydrate storage.

BRINK (1925) is of the opinion that differential pollen tube growth operates in combination with, or is caused by, differential carbohydrate metabolism. The fact that these four above mentioned characters do affect the nature or amount of carbohydrate storage might be considered as evidence in favor of such a view. We are of the opinion, however, that this is merely a coincidence; that distorted ratios in maize have been found mainly in endosperm characters only because such characters have been studied on a larger scale than any others. We feel certain that similar disturbances in the ratios of seedling and plant characters will also be found when these are studied on a greater scale. In fact, *high*, *low*, and *normal* ratios, apparently due to differential pollen tube growth, are now being obtained in a white seedling stock. (MANGELSDORF Unpublished).

The possibility, however, that differential carbohydrate metabolism may have some effect on pollen tube growth and that certain sporophytic characters may *per se* affect the rate of growth, is not denied. The marked deficiency of waxy seeds which BRINK (1925) obtained in a heterozygous waxy, homozygous sugary stock cannot, perhaps, be explained on the basis of gametophyte factors alone.

DISCUSSION

Selective fertilization has always offered an attractive explanation of peculiar results for which it was difficult to account by other means, and has frequently been called into account for unexpected departures from Mendelian ratios. Cuenot's yellow mice, CORREN'S aberrant ratíos in Melandrium, HERIBERT-NILLSON'S unusual segregation of certain characters in *Oenothera*, are examples[1].

EAST (1922) includes as one of the "Provisional Laws of Heredity" the statement that "there is no selective fertilization between complementary, compatible, functional gametes". This is probably true if the term

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[1] BRIEGER (1926) has recently summarized the cases in which selective fertilization might be used to interpret the results.

"fertilization" is confined to the actual fusion of the nuclei and a distinction is made between selective fertilization and certain other influences which disturb the random assortment of the gametes at the time of fertilization. For example; in plants or animals in which free-swimming, motile sperm come in contact with the egg in large numbers, there may be no discrimination by the female gametes in favor of certain of the male gametes, providing that all are compatible. There is no reason for believing, however, that all the sperm, in all cases, are equally capable of reaching the egg. It is possible that some classes are so handicapped as a result of their chromosomal constitution that though produced in full quota at gametogenesis, they are in minority at the time of fertilization.

In angiosperms an entire generation elapses between the time of gametogenesis and fertilization and the opportunity for differential elimination of certain classes is relatively large.

The pollen tube of angiosperms represents the remnant of the elaborate gametophyte generation in lower plants, in many of which this generation is of equal or greater importance than the sporophyte. In such plants there can be no question that the hereditary factors have almost, if not quite, as great an influence in the gametophyte as in the sporophyte generation. In fact, characters which have their expression in the gametophyte generation have recently been reported by WETTSTEIN (1924) in the mosses and ALLEN (1925) in the liverworts. In angiosperms the gametophyte generation has become greatly reduced and the hereditary factors which influence it have, no doubt, been correspondingly lessened, though not entirely eliminated.

Mendelian factors have been found to affect practically all stages in the ontogeny of the maize plant. The growing embryo, the endosperm, the resting stage of the seed, the young seedling, stature, chlorophyll development and reproductive processes, are all governed by genetic factors which may be unlike for different individuals. *It would be strange indeed if the gametophyte generation, brief though it has become, should be entirely deprived of the governing influence of various Mendelian factors.*

It is not surprising, therefore, to find in maize several Mendelian factors which have their expression in the gametophyte generation. These factors produce a definite physiological effect upon the gametophytes in which they occur, speeding up development in one case, reducing it in the other. They are transmitted from generation to generation in the same manner as any other Mendelian factors, occupy definite loci on certain chromosomes and show characteristic linkage and crossing over phenomena.

In general, the genetic factors which affect the gametophyte probably have little influence on ordinary Mendelian ratios in the sporophyte. In the female gametophyte, at least in angiosperms, they probably seldom cause any disturbance. In the male gametophytes they can have no effect as long as the parental sporophyte remains homozygous for the factors which influence the gemetophyte generation. Even when the gametophytes differ genetically, the favorable and unfavorable factors would, in the long run, tend to balance each other and no classes of gametes would be given a decided advantage. Only in occasional instances where a gametophyte factor produces a marked effect and is at the same time closely linked with a recognizable character in the sporophyte, is there any disturbance in the Mendelian ratios. Even here the total disturbance may not be noticeable when data from several generations are combined because a reversal of the linkage relations will cause deviations to balance each other.

Genetic factors affecting the gametophyte generation, giving certain gametes an advantage or handicap in accomplishing fertilization, need not contradict the Mendelian conception of random assortment. In some plants, however, they must be taken into consideration, as are linkage and lethal factors, in accounting for unexpected results.

SUMMARY

1. A stock segregating for defective seeds, de_1, regularly produces *high* ears with approximately 33 percent recessives, *normal* ears with 25 percent recessives, and *low* ears with 17 percent recessives.

2. These results are explained by assuming a factor, *Ga*, which has its expression in the gametophyte generation, stimulating the rate of pollen tube growth. *Ga* is linked with de_1 and in the coupling phase causes an excess of recessives; in the repulsion phase, a deficiency.

3. The cross of sugary × Rice Pop indicates the presence of a similar gametophyte factor linked with *Su* in the Rice Pop parent. The percentage of sugary seeds in F_2 is 16.2.

4. *Su* and de_1 are linked, with 39 percent crossing over, and it is probable that the gametophyte factor in both stocks is the same.

5. On this assumption it has been calculated that the *Ga* gametes accomplish fertilization 4.1 times as frequently as *ga* gametes and that the crossing over between *Ga* and de_1 is 24.3 percent; between *Ga* and *Su*, 21.2 percent.

6. That these values are approximately correct is indicated by WENTZ' data from a cross between de_{su}, a fourth character in this group, and a

stock with Rice Pop ancestry. This cross resulted in deviations approaching those predicted by the above calculations.

7. Gametophyte factors probably account, to a large extent, for the peculiar results obtained in mixed pollination experiments.

8. An analysis of KEMPTON's data indicates the presence, on the waxy chromosome, of a gametophyte factor causing distortions in the starchy, waxy ratio.

9. The action of the same factor is suggested as explanation of COULTER's peculiar ratios in aleurone color segregation.

10. The various ways in which gametophyte factors may operate and their effects on Mendelian ratios in the sporophyte are discussed in detail.

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UNIT CHARACTER INHERITANCE OF COLOR IN THE BLACK RAT, *MUS RATTUS* L

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INTRODUCTORY REMARKS

A presentation of certain facts concerning the inheritance of color in the Black rat, *Mus rattus rattus* L., and its allies is justified for at least two reasons. The inheritance of color variations in these rodents illustrates the type which may exist in essentially wild mammals. The extent of knowledge of the inheritance of similar variations in other rodents makes interesting comparisons possible.

The literature pertaining to the old-world rats in earlier times is not altogether clear. *Mus norvegicus* Erxleben, or *Mus decumanus* Pallas, commonly termed the Norway or brown rat, seems to have been confused with the members of the *rattus* group by several writers in spite of its distinctive characters and history. The *Mus rattus* group includes a considerable series of forms which are described at some length by HINTON (1919). As a group they are probably indigenous to INDIA. Extension of their range into Europe is evidenced by remains in pliocene deposits in LOMBARDY, in the quaternary near PISA, in pleistocene deposits on the island of CRETE, and in glacial times and with remains of the lake dwellers in western GERMANY and MECKLENBURG. At the present time the largest variety of forms occurs in southern ASIA. Ships have conveyed members of the group, however, to practically all parts. In the UNITED STATES well established colonies of the black rat, *Mus rattus rattus*, occur in northern localities, especially NEW ENGLAND. The roof rat, *M. rattus alexandrinus*, Geoffroy, is common in the southern states, especially along the seaboard.

Specimens of *M. rattus*, taken wild, exhibit a considerable range of color variability. The typical black rat on the one hand, is essentially black, while the roof rat is agouti dorsally, and creamy white ventrally. The variability within each of the types is also great. Certain subspecies and races from northern AFRICA, INDIA and CHINA are similar to the roof rat dorsally but show ventral pigmentation of a dusky or gray type. Such races resemble the Norway rat in coloration. Geographical isolation supplemented by mutation seems to have produced at least three distinct types in several localities.

MORGAN (1909) crossed the black rat with the roof rat. All of the resulting 32 animals were black. These mated *inter se* produced 4 black and 1 gray progeny. It was concluded that black is dominant over gray or agouti.

More recently several additional color variations have been reported which evidently occurred as mutations in wild colonies. PATTERSON (1920) described a cinnamon type which constituted approximately 7.5 percent of a population of the roof rat on two adjacent farms in TRAVIS COUNTY, TEXAS. HAGEDOORN and HAGEDOORN (1922) noted several variations in a hybrid population from several subspecies. Yellow, chocolate, waltzing, white tail-tip, and albinism were included.

CREW (1923) described a mutant of the black rat which occurred in warehouses of BRISTOL, ENGLAND in 1920. This form had a fawn and white coat and black eyes. It behaved as a Mendelian recessive character in crosses with the black. CREW stated that it was not the same form which HAGEDOORN described, for it did not have red eyes which darkened with age, as did their yellow type.

The writer (FELDMAN 1923) described the inheritance behavior of a black variety which was recessive or hypostatic to agouti. Also, in 1923 (FELDMAN 1923a) the knowledge of color inheritance in *Mus rattus rattus* and *M. rattus alexandrinus* was presented in a summarized form as far as the experiments had revealed at that time.

The purpose of this paper is to describe briefly the appearance of certain color varieties of these rats and to give the mode of their inheritance which the experiments have thus far indicated. They include black varieties (epistatic and hypostatic to agouti), agouti with white belly, agouti with gray or ticked belly, cream dilution and "blue" dilution. The nomenclature used in the descriptions is based on that of RIDGWAY (1912). Careful comparisons of the rats with his color standards were made in the daylight. The experimental evidence on the inheritance of the variations was gained through records of 943 individuals obtained in a series of different, controlled matings from 1922 to 1925 inclusive.

Opportunity is taken at this point to thank Doctors C. C. LITTLE and W. H. GATES for supplying live individuals of the black rat and roof rat respectively. The writer is indebted to Doctor W. E. CASTLE for his kindness in making the entire stock of the species available for investigation and for his helpful suggestions at various times.

DESCRIPTIONS OF THE VARIETIES

The original specimens of the black rat, *Mus rattus rattus*, were obtained from Doctor C. C. LITTLE who received them from Mr. P. C. HEALD of WILTON, NEW HAMPSHIRE. The four males and one female were very similar in appearance. Three of the males were successfully bred. The coloration of the black rat is typical of melanic forms. The mid-dorsal region is black and in some specimens is iridescent. The sides gradually shade from blackish mouse-gray to mouse-gray on the ventral surface. The six shades of neutral gray from dusky to pale also describe the color of this form, but slightly less accurately. On the sides and ventral surface of most individuals are scattered light hairs of cartridge buff. The skin of the ears, tail and feet is dusky mouse-gray. The eyes are black

Typical roof rats, *Mus rattus alexandrinus*, were sent to us by Doctor W. H. GATES from BATON ROUGE, LOUISIANA. They consisted of two young females which produced numerous progenies. This form has a typical agouti pigmentation. With the exception of the ventral surface, the pelage is composed mainly of hairs which are dark to light mouse-gray at the base and which are tipped with buff varying from ochraceous to pale ochraceous. Among the parti-colored hairs, completely dark and dark-tipped ones are scattered. The intensity of the black and yellow pigments is greatest on the mid-dorsal area, and becomes gradually lighter as the cartridge buff to white ventral surface is approached. This light area which comprises approximately one-fourth of the total, is sharply defined. The skin on the ears, tail and feet is mouse-gray in color and is thinly covered with buff-colored hair. The eyes are black.

In a shipment of rats of the *rattus* group received from Mr. H. C. BROOKE, a fancier of TAUNTON, ENGLAND, two additional color types were apparent and experiments revealed still another. Of three agouti females, two were typical of the roof rat coloration, agouti with light belly. The third, however, had a dark or ticked ventral coloring which resembled that of the Norway rat. This type reoccurred in the descendants of the female and other rats of the shipment. The dorsal and lateral coloration of the variety agouti with dark belly, is similar to the typical roof rat. The average individual is slightly darker, however, due to a larger proportion of hairs which are either entirely dark or are dark at the tip as well as at the base. The ventral hairs have a base of mouse-gray and an apex of cartridge buff. The skin at the extremities is very similar to that of the black rat. The eyes are also black. In this variety we evidently have the same coloration which is found in colonies of the wild subspecies.

The second new color type in the shipment from Mr. BROOKE was a cream variety. The coat in this form is typically cartridge buff to ochraceous buff on the mid-dorsal region, becoming lighter on the sides. The ventral surface is about six shades lighter than the mid-dorsal and is usually creamy-white. Approximately half of the cream animals have dorsal areas in which some of the hairs have light mouse-gray bases. A sooty appearance results. There is considerable individual variability in the intensity of the yellow and the intensity and extent of the sooty areas. The skin on the ears, tail, and feet is pallid mouse-gray colored. The eyes are black. This type is undoubtedly the one described by CREW, *loc. cit.*, and named by him *Mus rattus rattus* var. *brookei*, since our stock came from the same source as CREW'S.

There appeared in matings between agouti individuals and between agouti and cream, a black type. Breeding tests demonstrated that this is a new black variety which resembles the typical black rat in appearance, but has a different genetic relation to the other varieties.

In September, 1923, we received from Mr. BROOKE a "blue" variety. He has informed us that 4 "blue", 1 "blue-gray" and a number of black specimens were trapped on a boat at LIVERPOOL, ENGLAND, in the spring of 1923. The three males and two females which were sent to us were offspring of these animals. The color of this variety is dark mouse-gray dorsally shading to pallid mouse-gray on the ventral side. The skin at the extremities is mouse-gray. The eyes are black.

A survey of what is known regarding the origin of these types of *Mus rattus* favors the view that the agouti pigmentation with white belly which is characteristic of the roof rat is the primitive type. The melanism characteristic of the black rat, and the type which is gray with dark belly probably arose as mutations at some time sufficiently remote to give rise to subspecies. The second (recessive) black type occurred as a mutation in the roof rat in recent times. While the cream, *M. rattus rattus* var. *brookei*, and the "blue" variety are recent mutations of the black rat.

The five different types of these rats are represented in figure 1.

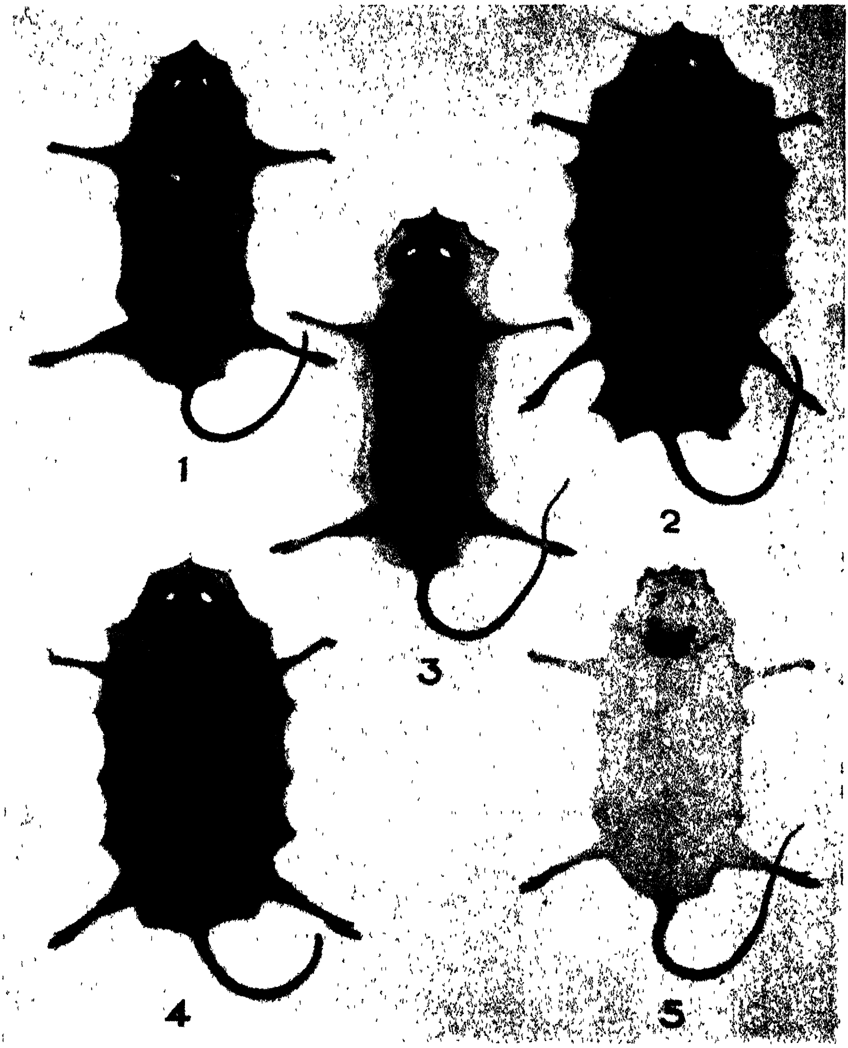

Figure 1. Skins representing five color types of, *Mus rattus.*

1. Typical Black rat, *Mus rattus rattus.*
2. Agouti variety with ticked ventral coloring.
3. Typical Roof rat, *Mus rattus alexandrinus.*
4. Dilute or "blue" variety.
5. Cream variety, *Mus rattus rattus* var. ***brookei.***

GENETIC RELATIONSHIPS OF THE VARIETIES

The experimental data indicate a simple Mendelian relationship of the factors producing the color varieties of this species. Thus far no indication of the linkage of any of the factors has been observed, although specific tests for it have not been made. The pertinent data are included in table 1.

TABLE 1

Summary of data on the inheritance of color in the Black Rat, Mus rattus rattus, and its allies.

NUMBER OF MATING	COLOR TYPES OF PARENTS	COLOR TYPES OF OFFSPRING					
		Black	Gray wh. ve.	Gray d ve.	Cream	Blue	Total
1.	Gray (white ventrally) *inter se*	5	12	1	9	..	27
2.	Gray (white ventrally)× gray(dark ventrally)	1	11	2	1	..	15
3.	Gray (white ventrally)× black(recessive)	3	20	..	2	..	25
4.	Gray (dark ventrally) *inter se*	5	..	13	3	..	21
5	Gray (dark ventrally)× black (recessive)	13	..	17	2	..	32
6.	Black (recessive) *inter se*	18	..	.	3	..	21
7	Gray (white ventrally)× cream	11	21	1	25	..	58
8.	Gray (dark ventrally)× cream	4	8	5	8	..	25
9.	Black (recessive)×cream	2	1	..	11	..	14
10.	Cream *inter se*	..	..	.	246	..	246
11.	Black (dominant)× Gray (white ventrally)	42	..	..	..	..	42
12.	F$_1$ from mating 11 *inter se*	46	16	..	..	..	62
13.	Black (dominant)×cream	36	..	..	..	..	36
14.	F$_1$ from mating 13 *inter se*	124	..	..	45	..	169
15.	F$_1$ from mating 11× black (recessive)	18	7	..	..	..	25
16.	F$_1$ from mating 13×cream	4	..	..	4	..	8
17.	Blue×blue	..	..	..	.	12	12
18.	Blue×gray (white ventrally)	7	..	..	..	..	7
19.	Blue×black (recessive)	14	..	..	..	..	14
20.	Blue×cream	17	..	..	..	..	17
21.	F$_1$ from mating 20 *inter se*	7	..	..	4	2	13

Three allelomorphs of the black extension factor exist. They are the melanism characteristic of the black rat, ordinary extension characteristic of the agouti pigmentation of the roof rat, and practical absence of black in the coat which exists in the cream or yellow variety. They may be represented by the symbols, E', E, and e respectively. Ordinary extension is epistatic to cream and the black is epistatic to both. Wild caught black rats, $E'E'$, mated to agouti roof rats, EE, produced black F$_1$, $E'E$ (see mating 11 in the table). The F_1 mated *inter se* produced approximately 3 black E': 1 agouti, E, young (mating 12). Likewise, the black rats, $E'E'$, mated to creams, ee, which were known to carry the agouti factor, produced all black, $E'e$, in the F_1 generation (mating 13). These F_1 mated *inter se* produced approximately 3 black E',: 1 cream, e, but no agouti colored, E, individuals (mating 14). When an F_1 male, $E'e$, was mated to a cream female, ee, a litter of 4 black and 4 cream young was produced. Rats which possessed ordinary extension, E, mated *inter se* produced a total of 141 offspring of which 20 were cream (matings 1 to 6). The deviation is accounted for by the fact that some of them were homozygous, EE. Animals of ordinary extension when crossed with creams produced 53 ordinary extension, Ee,: 44 creams, ee (matings 7 to 9). Again, the deviation from equality in this case was probably due to the homozygosity of some of the rats, EE. Creams mated to creams produced 246 young, all of which were cream (mating 10).

Individuals which are phenotypically ordinary extension, that is, of the composition, EE, or Ee, may show any one of three allelomorphic conditions of the agouti factor. These allelomorphs are, agouti with white belly, A^w, agouti with dark belly, A^d, and non-agouti, a. There is complete dominance of A^w, over A^d and a, and of A^d over a. The matings between these types may be seen to corroborate these statements (matings 1 to 6). A general excess of the dominant types is explicable by assuming the homozygosity of some of the animals involved for either A^w or A^d. Segregation of the recessive type or types was thus prevented in their particular litters. The E types when crossed with e types showed that the latter could possess any of the agouti factors in either a homozygous or heterozygous state.

The matings involving the "blue" dilution character, d, are very incomplete as yet. They demonstrate that it is recessive to normal intensity. They also show that the original "blue" animals were homozygous for the black extension factor, E'. All F_1 animals from matings between "blues" and agouti with white belly, recessive black, and cream rats were black, (matings 18, 19, 20). The observations of Mr. BROOKE referred to above indicate that the "blue" may occur in conjunction with agouti in the same individual, however. The matings show further that "blue" dilution is not an allelomorph of either the black extension series or of the agouti series. Crosses between "blue" and recessive members of both series produce in F_1 a reversion to the dominant type. Rats of two litters produced by mating the F_1 from the "blue" × cream cross *inter se* consisted of 7 black, 4 cream and 2 "blue" individuals. Two of the cream rats in these litters were double recessive $ee\ dd$ in nature. "Blue" appears to segregate, therefore as a typical recessive Mendelian character. "Blue" rats mated *inter se* produce only "blue" offspring.

With this survey of the genetics of color in these rats it is possible to state the factorial formulae of the animals which were used as follows:

1. Wild black rats, $E'E'\ DD$. (Their composition for the A factor has not been determined).[1]

2. Wild roof rats, $EE\ A^wA^w\ DD$.

3. Agouti rats with white belly, $EE\ A^wA^w\ DD$, $Ee\ A^wA^w\ DD$, $EE\ A^wA^d\ DD$, $Ee\ A^wA^d\ DD$, $EE\ A^wa\ DD$, and $Ee\ A^wa\ DD$.

4. Agouti rats with dark belly, EEA^dA^dDD, EeA^dA^dDD, EEA^daDD, and EeA^daDD.

5. Recessive black or non-agouti rats, $EE\ aa\ DD$, and $Ee\ aa\ DD$.

6. Cream rats, $ee\ A^wA^w\ DD$, $ee\ A^wA^d\ DD$, $ee\ A^wa\ DD$, $ee\ A^dA^d\ DD$, $ee\ A^da\ DD$, and $ee\ aa\ DD$.

7. "Blue" rats, $E'E'\ dd$. (Their composition for the A factor has not been determined.[1]

DISCUSSION

The genetics of the color varieties of *Mus rattus*, shows that two loci of the germ-plasm have undergone two changes or mutations each to produce the black extension series and the agouti series of allelomorphs. A third locus has mutated once to produce "blue" dilution. Three different chromosomes seem to be involved. In all probability additional loci have produced the cinnamon, chocolate and albino varieties described by other investigators At least two of these characters which mendelize, namely epistatic black and agouti with dark belly are characters which distinguish subspecies.

The unit characters of the black rat and its allies with which this paper deals are not unknown in other forms, especially in other laboratory rodents. There are varieties of the Norway rat, house mouse, guinea-pig and rabbit which resemble them very closely in appearance and hereditary behavior. It is unsafe, however, to assume that the characters of the black rat have exact homologues in the other forms.

Black extension allelomorphs occur in the guinea-pig and rabbit. In both species ordinary extension E, present in the wild agouti types, has two recessive allelomorphs. Tortoise-shell in the guinea-pig and the Japanese rabbit are mosaics of yellow and black which may be designated as e^t. They are recessive to E and dominant over yellow coat-color, e. In the rabbit a fourth allelomorph, epistatic black, E', exists. It is apparent that the series in *Mus rattus* has three allelomorphs in common with the rabbit, namely epistatic black, E', agouti extension, E, and cream, e. It also has the latter two in common with the guinea-pig. The mutation corresponding to the tortoise-shell and Japanese varieties has not been observed in this rat.

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[1] Since this paper was sent to press, F_2 individuals from matings of dominant black with recessive black, and "blue" with recessive black have been secured. The occurrence and proportions of agouti individuals with gray or ticked belly coloring indicate that the dominant black and "blue" rats carry that allelomorph of the agouti factor. They are, therefore, of the factorial composition, $E'E'\ A^dA^d\ DD$ and $E'E'\ A^dA^d\ dd$ respectively.

TABLE 2

Summary of allelomorphs of the black extension factor in the laboratory rodents.

	E'	E	$e^t m$	e
Rabbit	X	X	X	X
Guinea-pig	..	X	X	X
Norway rat	.	X	..	..
Black rat	X	X	..	X
House mouse	..	X	..	..

The agouti allelomorph series of the Black rat also shows considerable parallelism to that of the other rodents. The non-agouti mutation a, has been observed in the rabbit, guinea-pig, Norway rat, and house mouse. The guinea-pig, Norway rat and house mouse also exhibit agouti with dark ventral pigmentation, the third allelemorph of the Black rat. In the Norway rat the wild form has this type of agouti. A species of cavy, *Cavia rufescens*, studied by DETLEFSEN (1914) also shows this type of agouti in the wild state. The house mouse has in addition to the two types of agouti and non-agouti, an epistatic yellow allelomorph which has no counterpart in the series of other rodents. The black-and-tan variety of the rabbit, also an agouti allelomorph, has no counterpart among other forms. The three allelomorphic conditions of the agouti factor, A^w, A^d, and a of the Black rat are all present, therefore, in the guinea-pig and house mouse with the same order of dominance. Agouti with white belly, A^w, is lacking in the Norway rat, and the rabbit has no character corresponding to the agouti with dark belly, A^d.

TABLE 3

Summary of allelomorphs of the agouti factor in the laboratory rodents.

	A^y	A^w	A^d	a^t	a
Rabbit	..	X	..	X	X
Guinea-pig	..	X	X	..	X
Norway rat	..	..	X	..	X
Black rat	..	X	X	..	X
House mouse	X	X	X	..	X

As in the case of the other color characters of *Mus rattus*, "blue" dilution also occurs in other species. "Blue" varieties of the rabbit and house mouse are known which are Mendelian recessive characters to "normal" intensity. The factors producing the color varieties in these rodents indicate strongly the tendency of similar mutations or germinal changes to occur in closely related species. It is further evidence of a constant tendency of certain loci to mutate more frequently than others in any given species.

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STUDIES ON SELF-STERILITY VII. HEREDITY AND SELECTIVE POLLEN-TUBE GROWTH

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INTRODUCTION

A new genetic interpretation of self-sterility as manifested in *Nicotiana alata* Lk. and Otto *var. grandiflora* Comes and *Nicotiana forgetiana* Hort. (Sand.), or their hybrids, has been proposed recently (EAST and MANGELSDORF 1925). The detailed evidence on which this interpretation rests is given in the present paper.

Self-sterility appears to behave as a simple recessive when these species are crossed with the self-fertile species, *Nicotiana Langsdorffii* L. Their interaction is thus similar to that found by COMPTON (1912) in crosses between self-fertile and self-sterile strains of *Reseda odorata* L. The strains of Nicotiana homozygous for self-sterility behave as if their peculiar condition were controlled by several hereditary factors. Like combinations of factors produce like groups of plants in so far as their compatibility with each other is concerned, just as like factorial combinations which lie at the basis of morphological differences produce groups which are alike in those characters. Generally speaking, all members of a given self-sterility group are cross-sterile with each other reciprocally, but are cross-fertile with the members of every other group.

These distinctions in compatibility are due to hereditarily controlled reactions of such a nature that some pollen-tubes (compatible matings) show a rapidly accelerated rate of growth which permits fertilization in from three to five days,—depending mainly on the temperature, while other pollen-tubes (incompatible matings) show a slower and more nearly uniform rate of growth which ordinarily does not allow the generative nucleus to reach the micropyle before the flower withers and falls.

Obviously the differences between compatible and incompatible matings are quantitative, as were the differences between Mendel's tall and dwarf Pisum plants; nevertheless these differences are definite and concrete. There is no more reason for confusing the two types, as certain writers have done, than there is for confusing allelomorphs of numerous well demonstrated heritable differences of the kind commonly termed qualitative. For example, there are species of plants which produce colored flowers under ordinary horticultural conditions; but these plants may be carried through to the flowering stage under conditions which prevent the development of color in the petals, thus they will appear to belong to the same class as plants of the same species which lack the hereditary factor or factors necessary for color development and for which no yet discovered set of environmental conditions can promote color development.

Pollen-tube growth has been studied carefully in both compatible and incompatible matings of these self-sterile Nicotianas. Their growth-curves are characteristically distinct, and under the conditions most frequently met they do not overlap. In every slide studied a few pollen grains do not germinate, or, if they germinate, barely push through the stigmatic tissue. From the pedigree culture results on compatible matings wherein there is no deficiency of expected classes, we feel inclined to believe that this phenomenon is wholly somatic, unconnected with genetic differences. The remaining pollen-tubes in compatible unions show growth-curves having a very narrow variability; that is to say, fertilization in compatible matings is effected between the third and fourth day under all ordinary conditions, and there is probably not more than two hours' difference between the major and minor extremes. Furthermore variations in the environmental factors, such as moisture, plant age, etc., appear to advance or retard pollen-tube growth in these cases but slightly. Here only temperature changes seem to have an influence. In incompatible matings, on the other hand, external conditions have a marked effect. At the height of the flowering season, when pollinations are made on newly opened flowers, the average pollen-tube grows only about 8 mm in the length of time required by the pollen-tubes in compatible matings to traverse the whole length of the style (30 mm to 40 mm). It is noticeable, also, that the dispersion is much greater in the frequency distributions of incompatible matings than it is in compatible matings, though no precise determinations have yet been made. The rate of pollen-tube growth is especially affected by temperature variations and by the relative length of day and night, though it is difficult to determine the exact effect of each factor. One may say with some degree of confidence, however, that with vigorous plants, optimum temperature and light conditions will not affect mid-season incompatible matings sufficiently to produce confused results, though occasionally a small seed-capsule can be obtained.

Fortunately for the analysis of these sterility phenomena, there is another means of obtaining relatively well-filled capsules of seed from incompatible matings. The time during which pollen-tube growth can occur may be materially prolonged. Normally, at mid-season, the individual flower remains on the plant from 6 to 7 days if fertilization does not occur in the meantime. Toward the end of the flowering season this period, the flower life becomes longer. During the last two weeks of flowering the flowers remain alive 9 or 10 days, while one instance is recorded of 14 days.[1] Time of pollen-tube growth can also be extended by bud-pollinations. The old idea that stigmas are only receptive during a limited period, and particularly when they are secreting a substance assumed to promote pollen germination, certainly must have a limited application. In numerous species we have found that pollination of the young bud is fully as efficacious as pollination of the mature flower. In fact it may be that the secretions found in many so-called "ripe" stigmas are merely manifestations of cellular decomposition which occurs after the proper time for pollination. At any rate pollinations can be made 3 days before the flowers open in the species we are considering without any evidence of abnormal effects. In fact pollen-tube growth apparently is faster in bud pollinations than it is in end-season pollinations of open flowers. At least, incompatible matings made on buds 2 days before they open often yield full complements of seed, while similar matings made on newly opened end-season flowers which remain on the plants 3 days over the average, seldom yield more than one-half the normal amount of seed.

By taking advantage of this second means of ensuring fertilization after incompatible matings, inbred strains of our self-sterile hybrids between *N. alata grandiflora* and *N. forgetiana* have been carried through fourteen generations. The purpose of this procedure was to eliminate heterozygotes in case the factors controlling the behavior of self-sterile plants should be distributed in the ordinary manner at maturation. Should compatibility between self-sterile plants be brought about by the existence of constitutional differences in certain factors, then one ought to obtain near-homozygous populations after successive selfings or close matings which would consist of very few intra-sterile, inter-fertile classes. This, in fact, has been the case. Several families have been raised in which the plants all belonged to two intra-sterile groups, while a few families have been found to consist of only one such group. The purpose of this paper is to describe the reactions of these families.

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[1] SLATE, working in this laboratory, has determined recently that these times may be much greater in certain families.

PRESENTATION OF DATA

As soon as several families were obtained which consisted of but two intra-sterile classes, all possible combinations were made between them. Peculiar but consistent results were obtained. These results can be illustrated by calling the classes X, Y and Z. X crossed by Y gave classes Y and Z in approximately equal numbers. The reciprocal cross Y with X gave classes X and Z in about the same proportions. The same phenomena appeared when the other combinations were made. Class X crossed with class Z gave approximate equality of classes Y and Z, but class Z pollinated with pollen from plants of class X gave equal numbers of classes X and Y. Similarly class Y by class Z produced only X's and Z's in equal numbers, while class Z by class Y produced X's and Y's in equal numbers.

Thus it appeared that when individuals from any two classes were crossed, *two* equal sized classes resulted, but the class to which the female parent belonged was never represented.

These results may be interpreted by assuming that the cross-sterility here shown is controlled by three allelomorphs, S_1, S_2 and S_3; and that class X is S_1S_3, class Y is S_1S_2 and class Z is S_2S_3. If then it is further assumed that a plant affords stimulus only to pollen bearing sterility factors other than its own, all the data are satisfied.

When a Z (S_2S_3) female is crossed with an X (S_1S_3) male only the pollen bearing the factor S_1 is stimulated and functions. Segregation is of the ordinary type, but differential pollen-tube growth causes fertilization to be selective. The resulting progeny, therefore, consists only of the two classes S_1S_2 (Class Y) and S_1S_3 (Class X) in equal numbers. In the reciprocal cross, X (S_1S_3) female by Z (S_2S_3) male, the X female again affords stimulus only to gametes bearing factors other than its own, but this time it is the S_2 pollen. The resulting progeny is therefore solely of the two classes Y (S_1S_2) and Z (S_2S_3). Data from matings of this type are presented in tables 1 and 2.

Six crosses were made where the female parents had the constitution S_2S_3(Z) and the male parents had the constitution S_1S_3(X). Of the resulting plants 405 were classified by crossing with X, Y and Z testers. They fell into two classes; 201 were S_1S_2 while 204 were S_1S_3.

The reciprocal cross, S_1S_3 by S_2S_3, was made seven times. The progeny again fell into two classes, this time S_1S_2 of which there were 233, and S_2S_3 of which there were 205, a total of 438.

TABLE 1

Results of crossing Class S_2S_3 female with Class S_1S_3 male.

PARENTAGE	PROGENY		TOTAL
	S_1S_2	S_1S_3	
*L*1×8 *L*1*Q*2	52	59	111
*L*1×22 *L*1*Q*2	26	25	51
*L*1×23 *L*1*Q*2	39	43	82
*L*1×7 *R*1*L*1	56	47	103
*L*1×*Q*1	12	8	20
*L*1×*O*2	16	22	38
Total	201	204	405

Most probable expectancy, 202.5 : 202.5 Deviation, 1.5 P. E. 6.8

TABLE 2

Results of crossing Class S_1S_3 female with Class S_2S_3 male

PARENTAGE	PROGENY		TOTAL
	S_1S_2	S_2S_3	
8 *L*1*Q*2×*L*1	56	50	106
22 *L*1*Q*2×*L*1	39	40	79
23 *L*1*Q*2×*L*1	46	46	92
7 *R*1*L*1×*L*1	43	19	62
*Q*1 ×*L*1	21	18	39
*O*2 ×*L*1	8	16	24
*R*3 ×*C*13	20	16	36
Total	233	205	438

Most probable expectancy, 219 : 219 Deviation, 14 P. E. 7.1

In a second set of experiments, classes Z (S_2S_3) and Y (S_1S_2) were tested. Nine matings were made where S_2S_3 individuals were used as females and S_1S_2 individuals as males. From the resulting population, 493 individuals were tested, of which 248 belonged to the group S_1S_2 and 245 to the group S_1S_3. Ten reciprocal matings were made, S_1S_2 individuals being used as females and S_2S_3 individuals as males. Tests were made on 575 individuals coming from these crosses. Again there were two groups only—312 of class S_1S_3 and 263 of class S_2S_3. Here was found the greatest deviation from equality, a deviation slightly more than 3 times the probable error. The data are set forth in tables 3 and 4.

TABLE 3

Results of crossing Class S_2S_3 female with Class S_1S_2 male.

PARENTAGE	PROGENY		TOTAL
	S_1S_2	S_1S_3	
*L*1×10201	44	41	85
*L*1×*E*2	45	40	85
*L*1×1301	26	17	43
*L*1×21*R*1*E*2	23	21	44
*L*1×28*L*1*Q*2	42	58	100
8*R*3*R*1×*R*1	28	12	40
*C*13×*E*2	10	10	20
*L*1×*O*1	13	23	36
*L*1×*Q*2	17	23	40
Total	248	245	493

Most probable expectancy 246.5 : 246.5 Deviation, 1.5 P. E. 7.5

TABLE 4

Results of crossing Class S_1S_2 female with Class S_2S_3 male.

PARENTAGE	PROGENY		TOTAL
	S_1S_3	S_2S_3	
10201×*L*1	58	54	112
*E*2×*L*1	39	28	67
1301×*L*1	23	14	37
21*R*1*E*2×*L*1	22	22	44
28*L*1*Q*2×*L*1	56	35	91
*R*1×8*R*3*R*1	25	28	53
*N*1×*L*1	11	8	19
*Q*2×*L*1	50	30	80
*R*1×*C*13	12	20	32
*R*1×*L*1	16	24	40
Total	312	263	575

Most probable expectancy 287.5 : 287.5. Deviation 24.5. P. E. 8.1

In a third series of crosses, plants having constitutions S_1S_2(Y) and S_1S_3(X) were tested. Seven matings were made with S_1S_2 plants as females and S_1S_3 plants as males. From among the progeny, 396 individuals were classified; 189 belonged to group S_1S_3 and 207 to group S_2S_3. Six reciprocal matings were made, S_1S_3 plants being used as females and S_1S_2 plants as males. Testing out 317 of the resulting plants, it was found that 169 belonged to group S_1S_2 and 148 to group S_2S_3. The data for these crosses are found in tables 5 and 6.

TABLE 5

Results of crossing Class S_1S_2 female with Class S_1S_3 male.

PARENTAGE	PROGENY		TOTAL
	S_1S_3	S_2S_3	
*R*1×22 *L*1*Q*2	45	41	86
*R*1×23 *L*1*Q*2	38	51	89
*R*1×39 *R*1*L*1	30	43	73
*R*1×*R*3	14	26	40
*E*2×*R*3	21	9	30
*O*1×*O*2	23	17	40
*Q*2×*Q*1	18	20	38
Total	189	207	396

Most probable expectancy 198 : 198. Deviation, 9. P. E. 6.7

TABLE 6

Results of crossing Class S_1S_3 female with Class S_1S_2 male

PARENTAGE	PROGENY		TOTAL
	S_1S_2	S_2S_3	
22 *L*1*Q*2×*R*1	46	45	91
23 *L*1*Q*2×*R*1	40	36	76
39 *R*1*L*1×*R*1	28	20	48
*Q*1×*N*1	21	19	40
*R*3×*E*2	13	15	28
*R*3×*R*1	21	13	34
Total	169	148	317

Most probable expectancy 158.5 : 158.5. Deviation, 10.5 P. E. 6.

Altogether in these six experiments, 2,624 individuals were classified definitely by from one to fifteen pollinations made in various ways with test plants, without a single exception being found. In every cross the progeny fell into two groups of about equal size, and in no case was an individual produced belonging to the class represented by the mother. In addition, about 200 plants were grown which we were unable to classify precisely because unable to make the requisite pollinations. These plants are necessarily omitted from the records. They are reported as a matter of good faith; but it cannot be said that any one of them is an exception, because every plant given *all* of the necessary tests fell definitely into one or the other of the known classes.

Previously published data, recorded by Dr. E. S. ANDERSON, (1924) probably might have been added to the above results for the groups termed

X, Y and Z had we possessed the proper test plants to bridge the gap between his plants and our own. As things stand, all that can be said is that most of ANDERSON'S data are also concerned with three classes, and that our plants are descendants of the plants that he used. It is hardly to be questioned that our groups X, Y and Z are his groups A, B and C; but as to which is which we are not certain. Those of ANDERSON'S data that can be readily resolved into identical classes consistent with each other are as follows. Three crosses between A females and B males gave 25 B's and 22 C's. Three crosses between A females and C males produced 30 B's and 27 C's. One mating of C female with A male produced 7 A's and 9 B's. One mating between a B female and a C male gave 6 A's and 4 C's. And finally, the reciprocal of this cross, a mating of a C female with a B male, gave 4 A's and 6 B's. Thus in every case the group of the female parent was unrepresented, the progeny belonging to the other two groups, which were represented in approximately equal numbers.

A few other of ANDERSON'S figures come from the same family types, but the classes of the parents are unknown. In one case there was an apparent exception which was extremely puzzling. A family R (ANDERSON 1924, p. 32) consisted of 17 individuals belonging to class A and 20 individuals belonging to class C. Family X, which was supposed to have come from the reciprocal of the cross producing family R, gave results identical with those of family R. There were 10 individuals belonging to class A and 5 individuals belonging to class C. This situation was anomalous, for in no other case had reciprocal crosses produced plants belonging to the same two classes. Dr. ANDERSON luckily was able to solve the mystery because he had preserved the original seed envelopes. Several of the crosses used in his work had been made by an investigator who insisted on emphasizing male superiority by writing the male first in the laboratory records. Usually there was no confusion because the male and female signs were used, but in the particular case of family X, the seeming exception, these signs were left off though the figures were in the hand writing of the worker in question. According to the regular custom of this laboratory, therefore, family X was set down as the reciprocal of family R, when it actually was a duplicate of family R, and should have given the similar results that were actually obtained.

The behavior of plants carrying these three self-sterility allelomorphs, S_1, S_2 and S_3, was tested in other ways. Self-pollinations and incompatible cross-pollinations were rendered effective by pollinating buds about three days before they normally would open. Opportunity was thereby given for "like" gametes to fuse.

Family R1 was produced by selfing a plant belonging to class Y (S_1S_2). It consisted of 32 plants, of which 19 were typically class Y (S_1S_2), while 13 belonged to two new classes which were designated ZY and XY. Of the first class, ZY, there were 7 individuals. These plants were fertile as females with all classes except their own, but were sterile as males with classes Z (S_2S_3) and Y (S_1S_2) as well as with their own class. The 6 members of the second class, XY, were fertile as females with all classes except their own, but were sterile as males with class X (S_1S_3) and Y (S_1S_2) as well as with their own class.

Another class Y (S_1S_2) plant selfed, produced 39 individuals (Family Q2), of which 24 proved to belong to class Y, 14 to class ZY and 1 to class XY.

According to the selective pollen stimulation hypothesis class Y (S_1S_2) when selfed in the very young bud yields approximately 1 S_1S_1 (Class XY) + 2 S_1S_2 (Class Y) + 1 S_2S_2 (Class ZY). The S_1S_1 (XY) plants are fertile as females with

(a) Class Z (S_2S_3) because they accept both S_2 and S_3 pollen,
(b) Class X (S_1S_3) because they accept S_3 pollen, and
(c) Class Y (S_1S_2) because they accept S_2 pollen.

On the other hand, they are fertile as males with class Z (S_2S_3) because Z accepts S_1 pollen, but are sterile with both classes X (S_1S_3) and Y (S_1S_2) because neither of these classes accepts S_1 pollen.

Similarly individuals belonging to Class ZY (S_2S_2) are fertile as females with classes X, Y and Z, but are sterile as males with classes Y and Z.

Two other families were produced from this type of mating, but this time two different Y (S_1S_2) plants were crossed with each other reciprocally. The first cross (E2 × R1) yielded 17 class Y plants plus 5 class ZY plants plus 1 class XY plant. The reciprocal cross (R1 × E2) yielded 21 class Y plants plus 12 class ZY plants plus 6 class XY plants. Reciprocal crosses in this case, therefore, gave similar results as might be expected. The collected results from mating S_1S_2 plants together are found in table 7.

The data from these four families, each produced by mating S_1S_2 plants together, show clearly that there is a deficiency of S_1S_1 plants. There were 14 S_1S_1 plants, 81 S_1S_2 plants and 38 S_2S_2 plants. Apparently there is no marked selective fertilization, because there are just about one-half as many S_2S_2 plants as there are S_1S_2 plants. If heterozygotes were formed to the prejudice of homozygotes when there is equal opportunity for fertilization, it would seem as if there should be a great preponderance of S_1S_2 plants. This fact can be explained either by assuming that the S_2

pollen grows slightly faster than the S_1 pollen, or by S_1S_1 homozygotes being weaker than S_2S_2 homozygotes. Both of the homozygous classes are indeed weaker than the heterozygous class; but since they show practically the same vigor from time of germination to maturity, we are inclined to believe that the first postulate is correct. Briefly S_2 pollen grows slightly faster than S_1 pollen. Several additional critical crosses were made involving the homozygous class XY (S_1S_1), the results from which are strictly in accordance with hypothesis.

A homozygous S_1S_1 plant, 9 R1, was selfed in the young bud, giving us Family FA consisting of 31 plants. Each of these plants belonged to the class of the mother (S_1S_1).

TABLE 7

Results from mating Class Y (S_1S_2) plants together.

MATING	RESULT		
	S_1S_1	S_1S_2	S_2S_2
*R*1 (self)	6	19	7
*O*2 (self)	1	24	14
*E*2×*R*1	1	17	5
*R*1×*E*2	6	21	12
Total	14	81	38

Second, two families were produced by crossing the same plant as above, 9 R1, a homozygous S_1S_1, with a plant O2 belonging to class S_1S_3. Family FB was the result of a normal pollination, Family FC was the result of a pollination made in the young bud. Since by hypothesis the female was S_1S_1 and the male S_1S_3, it is to be expected that only S_3 pollen would function in either case. That is to say, S_3 pollen sufficient to fertilize all the ovules would reach the ovary before any S_1 pollen would arrive. This is what happened. In Family FB the 24 plants tested all proved to belong to the one class S_1S_3, while in Family FC the 32 plants tested all proved to belong to the same class S_1S_3. Thus it seems that selective pollen-tube growth is a strictly individual reaction between the pollen and the style. The growth of a large group of compatible pollen-tubes does not appear to produce anything in the nature of a secretion which *materially* accelerates the growth of incompatible pollen-tubes.

On the other hand, either a *slight* acceleration to the growth of an incompatible pollen-tube may be furnished by the growth of a number of compatible pollen-tubes, or else pollen-tube growth in a *very* young bud may follow a different curve from that followed in the mature flower. For

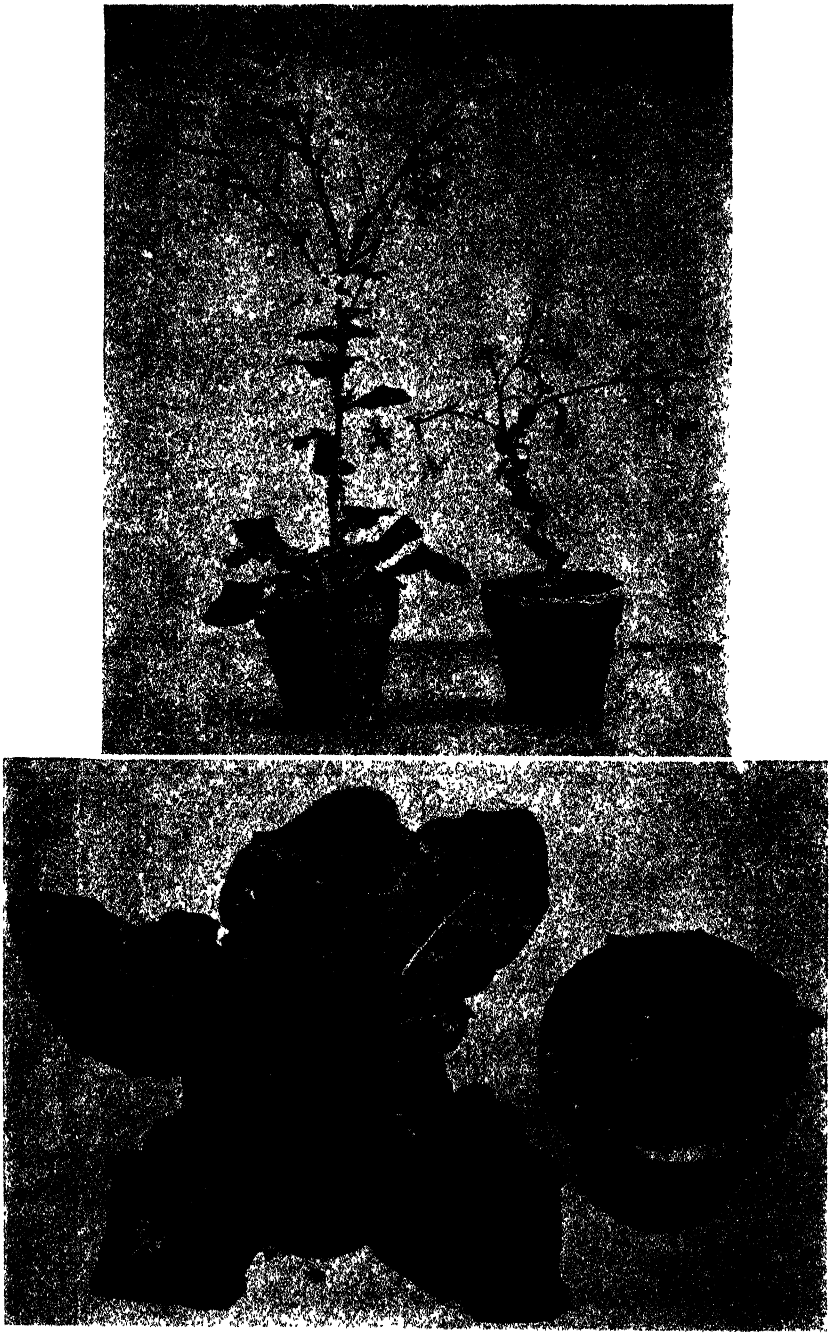

FIGURE 1. Plants of *Nicotiana Sanderae* Hort. showing the effect of the S_3 sterility factor in the homozygous condition At left, S_1S_3 plants; at right, S_3S_3 plants.

example, normally such a mating as S_1S_2 female by S_1S_3 male gives only the two classes S_1S_3 and S_2S_3. But in one case such a cross made in the *very young* bud, the style being still very short, gave 13 S_1S_3 plus 13 S_2S_3 plus 4 S_1S_2 plus 4 S_1S_1. The same cross made on a mature flower gave 26 S_2S_3 plants and 14 S_1S_3 plants. That this type of phenomenon is not unique is shown further by the results obtained when a S_2S_3 plant (L1) was pollinated in the very young bud stage with pollen from a S_1S_3 plant (O2). The resulting population consisted of 21 S_1S_3 plus 15 S_1S_2 plus 11 S_2S_3 plus 13 S_3S_3. Since in this case an almost equal number of compatible and incompatible pollen-tubes reached the ovary, we are inclined to suggest that the growth-curves of the two types did not have the opportunity to show extreme differentiation because of the relatively short distance traversed. This point is being determined and will be reported upon later.

The final and most critical test of the hypothesis involving S_1S_1 plants was the pollination of two different flowers of the same individual, 9 R1 (S_1S_1), with pollen from plants belonging to classes Y (S_1S_2) and ZY (S_2S_2) respectively. Here are two different crosses, the first being class XY by class Y, the second being class XY by class ZY; yet by hypothesis only S_2 pollen should function in both cases, and all resulting plants should belong to class Y (S_1S_2).

Family FD consisted of 24 plants resulting from the mating 9 R1 (S_1S_1) by R1 (S_1S_2). All belong to class Y (S_1S_2). Family FF also consisted of 24 plants resulting from the mating 9 R1 (S_1S_1) by 19 Q2(S_2S_2). Again all belonged to class Y (S_1S_2).

Crosses involving the homozygous class ZY (S_2S_2) gave similar results. Plant R1E2 (S_2S_2) when selfed, produced 31 plants all belonging to the one group S_2S_2. Again this homozygous S_2S_2 plant, 4 R1E2, was crossed with pollen from R1 (S_1S_2) producing Family ED, and with pollen from 23 O2 (S_1S_1) producing Family EF. Cross $S_2S_2 \times S_1S_2$ yielded 24 plants all belonging to class S_1S_2, while cross $S_2S_2 \times S_1S_1$ also yielded 24 plants all belonging to class S_1S_2, as was expected.

A smaller series of tests were made with the heterozygous classes X (S_1S_3) and Z (S_2S_3) simply because the apparently lethal character of the homozygous class S_3S_3 diminished the opportunity for producing interesting combinations. But it now happens that the peculiarities of the S_3S_3 group makes it the most interesting of all. For this reason, the experiments with S_1S_3 plants and with S_2S_3 plants will be discussed together.

Two S_1S_3 plants were selfed. The first, O2, produced 30 plants of group S_1S_3 and 13 plants of group S_1S_1; the second, R3, produced 27 plants of group S_1S_3 and 7 plants of group S_1S_1. Altogether 57 plants of class S_1S_3 and 20 plants of class S_1S_1 were obtained.

Likewise, when plant C13 (S_2S_3) was selfed, the resulting population consisted of 17 S_2S_3 plants and 12 S_2S_2 plants.

In none of these populations was a single S_3S_3 plant discovered; yet because there was no marked excess of heterozygotes over the types of homozygotes found, it seemed unlikely that the heterozygotes were produced wholly by the union of S_3 ovules and S_1 or S_2 pollen, as the case might be. It seemed more probable that S_3 pollen had reached the ovaries and had functioned in the production of heterozygotes. For this reason we concluded in our former paper (EAST and MANGELSDORF 1925) that S_3 is lethal when in the homozygous condition.

This conclusion is not wholly correct. In all three of these populations a number of abnormal seedlings appeared, apparently affected by root-rot. They were removed from the seed pans in order not to contaminate the healthy plants. Most of them died at an early age after showing typical root-rot symptoms. A few, however, grew slowly, came to maturity, and proved to belong to class S_3S_3. Every S_3S_3 plant found was of this type. The truth is, therefore, that each of these three populations yielded an unknown percentage of S_3S_3 plants.

While it is not strictly true that S_3 is lethal when in the homozygous condition, it certainly has lethal tendencies. During the winter months, with their short days, it is almost impossible to bring S_3S_3 plants to maturity. When grown during the spring and summer months they are somewhat more viable, but even then they seldom live long enough to produce more than a few flowers. The growth habit is peculiar to themselves. The leaves are more crinkled and the tips more rounded than their normal sisters, and each leaf has a characteristic outward roll, reminding one of spinach. Even fifteen-day-old seedlings can be separated unerringly by their appearance. The flowering branches of the older plants are usually weak and drooping. Efforts to keep plants alive long enough to mature seed capsules have thus far been unsuccessful. The flowers are normal, however, and the pollen in most cases is good. The plants can be classified, therefore, by their reactions as males.

The root-system is always reduced, as if by the action of root-rot. It is barely possible that the whole appearance is the result of a high susceptibility to root-rot, therefore; but the fact that most varieties of plants showing a root-rot infection sufficient to change their whole appearance do not live to maturity, makes it more probable that the morphological changes are due to the direct action of the factor S_3.

It is also possible, of course, that the type comes from the action of another hereditary factor closely linked to S_3, but thus far no crossovers have been found.

Among all of these tests, one can hardly imagine more critical experiments than those made on the homozygous families. Anyone who has puzzled his brain over the peculiar complications emerging from crosses between self-sterile plants will readily appreciate the weirdness of the following results. Here was a family of plants, XY, all inter-sterile. Another family of plants, ZY, was similarly inter-sterile. But XY individuals were fertile with ZY individuals; and reciprocal crosses for the first time produced similar results, for hitherto we had been dealing with matings between heterozygous types where the results of reciprocal matings were unlike. Every plant produced, either by cross XY × ZY or by cross ZY × XY, proved to belong to class Y. That is to say, these F_1 plants all belonged to a single sterility group, and this group was not the same as the two groups to which the parents belonged. Yet when tested as males on the F_1 plants, the two parental types, which had previously been shown to belong to separate classes, might readily have been classified together as one group. In other words, the parental classes XY and ZY were both sterile as males with all plants of their progeny, group Y. On the other hand, XY plants were fertile as females with pollen from Y plants and produced families of all Y plants. Likewise ZY plants were fertile as females with pollen from Y plants and again produced families of all Y plants,— a most astonishing result.

These seemingly anomalous and contradictory results are precisely what is to be expected by hypothesis. Class XY is S_1S_1, class ZY is S_2S_2. These two classes are fertile reciprocally and produce plants all of which belong to the one classY $=S_1S_2$. Each of these S_1S_2 plants is sterile to the pollen of the parental classes; in the first case because S_1 pollen is not stimulated, and in the second case because S_2 pollen is not stimulated. On the other hand S_1S_1 plants are fertile with the pollen of S_1S_2 plants because S_2 pollen is stimulated and results in S_1S_2 plants being formed, while S_2S_2 plants are fertile with the pollen of S_1S_2 plants because S_1 pollen is stimulated and results in S_1S_2 plants being formed.

DISCUSSION

The analysis of the behavior of the self-sterile Nicotiana plants which has been made in this paper, does not complete even the factorial interpretation for Nicotiana. Another member of the same allelomorphic series, S_4, has been discovered and proved to be distinct from factors S_1, S_2 and S_3.

Three other factors have also been found in material from different sources which may prove to be different from those already described, but critical tests on them have not yet been completed. Nor is it certain that only one locus is involved in the control of self-sterility.

It is hoped that these matters can be more or less definitely settled within the next few months, and that some light can be thrown on the probable number and distribution of factors within these species. But even if matters should thus work out according to our most optimistic expectations, there is still much doubt as to the physiological meaning of the genetic results. It is clear that there is an interaction between the pollen-tubes and the tissues of the styles due to their genetic composition. But it is not certain whether *similar* genetic formulae cause inhibited growth, or whether *different* genetic formulae cause accelerated growth. This distinction may seem like a play on words, but there is a real difference which can be explained only when we know whether more than one locus is involved in controlling self-sterility and what are the exact types of the pollen-tube growth curves.

Some of these problems together with the question as to whether the interpretation of self- and cross-sterility here proposed can be extended to other groups of plants will be discussed in a later paper.

SUMMARY

1. In the populations of Nicotiana used in these studies the behavior and inheritance of self-sterility and of its corollary, cross-sterility, are shown to be determined by three allelomorphic sterility factors S_1, S_2 and S_3.

2. The action of these sterility factors is such that the growth of pollen tubes carrying a given factor is inhibited in the styles of plants carrying that factor.

3. As a result reciprocal crosses between plants having one of the sterility factors in common give unlike progenies, and the sterility group to which the female parent belongs is always absent in the progeny. S_1S_2 ♀ × S_1S_3 ♂ gives equal numbers of groups S_1S_3 and S_2S_3, while the reciprocal cross S_1S_3 ♀ × S_1S_2 ♂ gives equal numbers of groups S_1S_2 and S_2S_3. The other possible combinations give analogous results.

4. Populations produced by selfing in the young bud stage show the expected segregation. Group S_1S_2 selfed gives groups S_1S_1, S_1S_2 and S_2S_2. Group S_1S_3 selfed gives groups S_1S_1, S_1S_3 and S_3S_3. Group S_2S_3 selfed gives groups S_2S_2, S_2S_3 and S_3S_3.

5. Tests of various crosses between both heterozygous and homozygous plants have given the sterility and fertility reactions expected by hypothesis.

6. One of the allelomorphs (S_3) in the homozygous condition produces characteristic abnormalities in habit of growth.

7. New material now being tested has given evidence of additional sterility factors. Complete tests have been made on only one type. It proves to be a fourth allelomorph, S_4, belonging to the same series as those previously described.

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THE MATERNAL INHERITANCE OF A SEX-LIMITED LETHAL EFFECT IN *DROSOPHILA MELANOGASTER*

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SUMMARY

The present paper describes the maternal inheritance of a lethal effect in *Drosophila melanogaster* which leads to the early death of a majority of female zygotes, and which in consequence gives rise to an average sex-ratio among adults of one female to 5.5 males. The effect may be transmitted both through the male and through the female; the genetic basis involves a recessive gene in the second chromosome group. The missing females probably die in the egg stage. The maternal effect may be attributed to an influence of the chromosomal composition of the mother upon the eggs which will give rise to the offspring exhibiting the lethal ratio previous to the time when these eggs leave the mother's body. The presence in such eggs, after fertilization, of the female-determining chromosomal complex as contrasted with the presence of the male-determining chromosomal complex is responsible for the differential death rate.

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[1] The data included under these two headings were obtained while the writer was Nationa Research Fellow in Zoology at COLUMBIA UNIVERSITY.

HISTORICAL

It has long been known, as shown for example by the extensive work on reciprocal species crosses in echinoderms and teleosts, that the developing embryo may for a longer or shorter period exhibit characters typical of the race from which the egg was derived. But an intrinsic difficulty from the genetic standpoint in much of this work is that the hybrids cannot be raised to maturity and bred. It is only when succeeding generations are obtained that we have the data necessary for determining whether a given case of maternal inheritance is quite independent of the nucleus or is the result of a deferred nuclear influence. The intimate relation of the egg with the mother's body, as well as the fact that preceding reduction it contains the chromosome complex of the mother, afford a basis for the latter type of maternal inheritance.

In regard to those characters which are shown to be determined by the genic composition of the mother and to which in consequence the term "maternal inheritance" is applied, it should be noted that this term is not happily chosen. For although the characters are immediately fixed by the constitution of the mother, they are just as definitely inherited from the father as from the mother, as becomes apparent in following generations.

The work of TOYAMA (1912 and 1913), TANAKA (1924), and others has demonstrated that cases of maternal inheritance shown by egg characters (shape, color, voltinism) of the silkworm moth may be explained on the basis of segregating genes. The color characters, which have been most extensively studied, depend upon the yolk (deposited while the egg is still part of the maternal body), the shell (derived from the epithelium of the oviduct), and the serosa. UDA (1923) has recently questioned the conclusions of TOYAMA regarding the transmission of brown versus slate, dependent upon serosa pigmentation; but it is clear from UDA's paper, as has been pointed out by TANAKA (1924) and PELLEW (1925), that his data, as well as the discrepancies in TOYAMA's papers, are readily explainable on the basis of an influence of the maternal genetic composition combined with an influence of the composition of the embryo itself.

STURTEVANT (1923) has suggested that the data of BOYCOTT and DIVER (1923) on the heredity of the direction of coiling in the snail, *Limnaea*, might be interpreted in terms of maternal inheritance. The recent paper of DIVER (1925) indicates that in addition to the parental nuclear influence the genetic composition of the zygote itself may be a further factor; there are possibly other as yet undetected influences at work in determining this character.

The studies of GOLDSCHMIDT (1924) on the transmission of the larval pattern resulting from crosses of geographic varieties of the gypsy moth, *Lymantria dispar*, have led him to conclude that besides the hereditary differences dependent upon segregating genes there is evidence of further differences which depend upon the plasma furnished by the egg and by the spermatozoon. In this respect, as is to be expected, the egg is the more influential. It may be recalled that GOLDSCHMIDT'S work on intersexes in this form shows that the "femaleness" factors are inherited, without paternal influence, exclusively from the mother. They were previously (1916 and 1917) attributed to the cytoplasm of the egg. It is now (1922 and 1923) suggested that they are transmitted by the "Y" or "W" chromosome and that a deferred effect is exhibited. This latter explanation is based upon the appearance of individuals assumed to be non-disjunctional.

Occasional characters are reported for *Drosophila melanogaster* whose expression shows more or less influence of the unreduced genetic composition of the mother. Such a maternal effect was reported by MORGAN (1912 and 1915) for rudimentary: by LYNCH (1919) in a study of a number of mutant forms showing partial or complete sterility in certain crosses; these forms include fused, rudimentary, morula, dwarf, and reduced bristles: and by MOHR (1925) for a third-chromosome recessive minute. An interesting point established by Miss LYNCH is the partial restorative effect upon viability at some stage of the presence of the normal allelomorph (for example of fused). This restorative effect may be exercised: (1) by the normal allelomorph of fused when present in the unreduced egg, even though the mature egg may contain fused and may be fertilized by a spermatozoon carrying fused, or (2) by the introduction of the normal allelomorph of fused by the spermatozoon.

Typical egg characters in *Drosophila melanogaster* are known to be determined by the genetic composition of the mother. MOHR (1922) found that the singed gene causes the eggs of homozygous females to be short and deformed. WARREN'S study (1924) of egg size in various races indicates that this character is dependent upon genes in all four linkage groups and that it is determined by the genetic constitution of the mother exclusively. This is to be expected since the size of the egg is fixed before the entrance of the spermatozoon. An interesting character of this type has recently been found by J. C. LI (unpublished work from the Columbia laboratory) in the eggs of Minute-l females. These eggs are shorter and rounder than normal eggs and are considerably more transparent so that developing structures are readily observed.

ACKNOWLEDGMENTS

The work, a report of which follows, was conducted under the direction of Professor S. J. HOLMES of the Department of Zoology of the UNIVERSITY of CALIFORNIA. Laboratory accommodations were extended by the Division of Genetics. I am grateful also to the following for stocks and for suggestions: Professor H. J. MULLER of the UNIVERSITY of TEXAS, Professor R. E. CLAUSEN of the UNIVERSITY of CALIFORNIA, and Dr. C. B. BRIDGES of COLUMBIA UNIVERSITY.

FIRST APPEARANCE OF THE LETHAL

The original culture exhibiting a sex-count with the low proportion of females appeared in February, 1923, among the progeny of a cross involved in an experiment designed to measure the rate of lethal mutation in the X-chromosome of *Drosophila melanogaster.* Three generations previously a female from wild type stock had been mated to a male from eosin-vermillion-forked stock. (Eosin, vermilion and forked are sex-linked recessives with the following symbols and loci: eosin, w^e, 1.5: vermilion, v, 33.0: forked, f, 56.5 BRIDGES, 1921). A phenotypically wild female from this cross, with one X-chromosome of the wild type, the other containing eosin, vermilion and forked, was mated to an eosin-vermilion-forked male. A similar selection was made among the progeny for the parents of the next generation; these were the parents of the lethal culture.

The expectation in such a cross is approximately equal numbers of males and females and approximately equal contrary classes. By the procedure of mating normal-appearing females with eosin-vermilion-forked sibs in successive generations, a new sex-linked lethal may be detected. For not only are there disturbances in the sex-ratio resulting from the death of males, but one class of each pair of male contrary classes is markedly smaller than the other. Thus a measure of the rate of lethal mutation in the X-chromosome may be obtained. (MULLER and ALTENBURG 1919).

TABLE 1

A. The original lethal culture, $\underline{w^ev}f$ ♀ × w^evf ♂. *A deficiency in the male classes with the eosin end of the maternal* w^evf *chromosome is shown.*

w^evf		+		w^e		vf		w^ev		f		w^ef		v	
♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
0	8	1	44	0	10	0	19	0	4	0	9	0	0	0	2

The original culture gave, as table 1A shows, an unexpected count of one female and 96 males. Moreover, there seemed also to be discrepancies in the male classes receiving the eosin end of the eosin-vermilion-forked chromosome of the mother. The one female was sterile, for she produced no offspring by either an eosin-vermilion-forked sib or by a fresh wild type male. Such sterility is frequent in the lethal strain. The explanation of the deficiencies shown by the male classes receiving the eosin end of the mother's eosin-vermilion-forked chromosome is not clear. They are probably not purely "fortuitous". That they are not due to the heterozygous presence in the mother of a sex-linked *semi*-lethal in this region is shown by the fact that four cultures, producing grandsons of the eosin-vermilion-forked males did not show such deficiencies. The totals show the following male contrary classes: 85 wild type, 60 w^evf; 23w^e, 30vf; 29w^ev, 22f; 5w^ef, 6v; 262 females were produced. That the discrepancies in the eosin-containing male classes of the first lethal culture are not necessarily correlated with the production of the low female count is demonstrated by the following: (1) The lethal strain was derived from a non-crossover wild type male of this culture. (2) Later lethal counts were obtained from crosses of eosin-vermilion-forked males by females one of whose X-chromosomes contained eosin, vermilion and forked (the other X-chromosome was wild type), and these counts did not present comparable deficiencies in the male classes containing eosin. This is shown by table 1B.

B. Further crosses of $\underline{w^evf}$ ♀ × w^evf ♂ *which give lethal counts. The lethal has been introduced from the lethal strain. No significant deficiency is shown in the male classes with the eosin end of the maternal* w^evf *chromosome.*

	w^evf		+		w^e		vf		w^ev		f		w^ef		v	
	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
	8	25	3	35	1	17	2	18	1	5	1	8	0	2	0	0
	0	21	1	21	0	7	0	9	0	2	0	6	0	1	0	2
Total	8	46	4	56	1	24	2	27	1	7	1	14	0	3	0	2

The most plausible explanation of the irregularities in the contrary classes of table 1A involves the assumption that a new sex-linked lethal, quite a different lethal from that which kills females, arose in the mother of the culture. This new male-affecting lethal was supposedly located in the eosin end of the eosin-vermilion-forked chromosome and was not present in all of the unreduced eggs. The latter would necessarily be true since those of the surviving eosin-vermilion-forked sons which were tested did not transmit such a lethal. Sex-linked lethals of this type are ordinarily kept by breeding the heterozygous females. The procedure was impossible in this case because these females were killed by the maternally inherited lethal. That is to say, the only individuals which would be expected to survive and show evidence of such a sex-linked lethal were killed by another lethal. It is obvious that this hypothesis remains tentative, since of necessity it is not amenable to test.

It should be noted concerning the cultures of table 1B that the lethal counts given are of F_2 offspring of a cross of an eosin-vermilion-forked female from the mutation rate experiment mentioned above, by a lethal male. The grandmother consequently is from the strain from which the female-killing lethal originally was derived. The interpretation of maternal inheritance employed later in the paper implies that if out-crosses are made of lethal males to non-lethal strains and the progeny are inbred, the female-deficient counts should not be produced until the F_3 individuals are obtained. However, if both parents should contribute the lethal, such counts might appear before F_3. Since the grandmother of the individuals included in table 1B was from the strain from which the lethal was derived, it is reasonable to assume that she may have been heterozygous for one or more genes concerned. If so, these cultures show no unexpected results. Of the 256 cultures which exhibit the lethal count (included in figure 1) these two alone show characteristics at first sight (and only at first sight) not compatible with the interpretation of maternal inheritance.

The exact conditions under which the lethal originally arose are unknown. Judging by its subsequent behavior, which shows that the mother of a lethal count must be homozygous for a second chromosome recessive. it seems most probable that this recessive was already present in the eosin-vermilion-forked stock which appears in the pedigree of the strain. If so, the several intervening generations produced the genetic combination conducive to the appearance of the female-deficient ratio.

CHARACTERISTICS OF THE LETHAL

The most outstanding feature of the lethal is, of course, the low proportion of females produced. The total number of flies from all cultures known to exhibit the lethal ratio gives 3,982 females and 21,821 males; thus the average sex-ratio is 1 female : 5.5 males. However there is a great variation in the sex-ratio produced by different cultures; for although a considerable proportion of the cultures produce very few females, or none, in others the sex-ratio more nearly approaches equality. This variation is shown by figure 1.

It should be stated concerning the cultures included in figure 1 that there has been an appreciable increase since the discovery of the mutant in the number of females produced per bottle. Of the cultures producing no daughters, a relatively larger proportion appeared early in the work; the large number of cultures giving 20 to 30 percent females came late. Since the environment was the same at these two periods the effect may be attributed to selection. It is obvious that the strain would be perpetuated by matings from those bottles which, although showing definite lethal ratios, gave female offspring. Modifiers would thus be selected for whose tendency would be partially to counteract the lethal effect.

A few cultures producing sex-ratios intermediate between 1 female : 2 males and 1 female : 1 male were excluded from the totals and from figure 1 because, although several of these were shown by breeding tests to contain the lethal (there were about 6 of these; they would not substantially alter the sex-ratio of 1 female : 5.5 males), others were not so tested, and some of these latter undoubtedly did not represent lethal counts. Experience with non-lethal lines demonstrates that occasional fortuitous ratios of this type are produced, particularly when the number of offspring is not large. The tendency of such of these cultures as may have been lethal slightly to alter the sex-ratio would, moreover, be at least largely offset by the inclusion of cultures undoubtedly representing lethal counts which were excluded on the basis of their very low production.

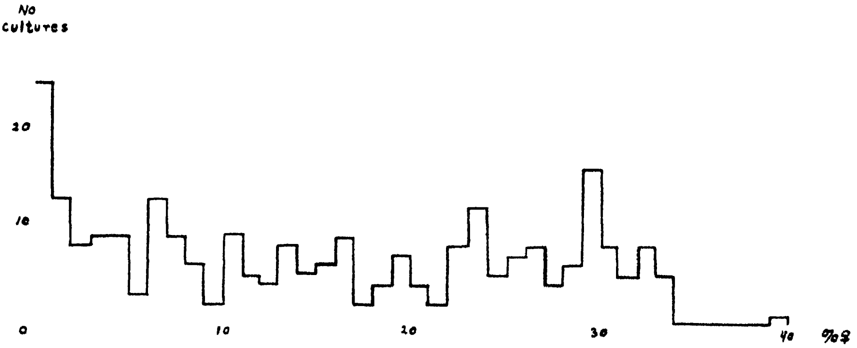

FIGURE 1. Variation in the female percentage of the total number of flies in cultures exhibiting the lethal count.

Especially unfortunate attributes of the lethal strain are its high sterility and its low productivity. Large numbers of individuals produce no offspring whatever, and this seems to be due to sterility in both sexes. When offspring are produced, the numbers frequently are very small. Broods are produced, however, as an examination of the tables shows, which are large in size. The factors of low productivity and of high sterility have made the collection of data difficult. Indeed it is probable it would not have been considered worth while to preserve the strain, had it not been for a peculiarity of behavior indicating maternal inheritance.

Other characters of the lethal strain include various morphological abnormalities. Chief among these is the presence in all lethal cultures, of broken abdominal bands (at least since this character was discovered very early in the work). There is considerable variation in the extent to which this character is exhibited. Most, if not all, broken bands seem to be dependent upon a tendency to reduction in the dorso-lateral plates; in some individuals one of these plates is completely suppressed. Probably at least some of the sterility of the strain is correlated with these broken bands, for frequently the external genitalia of broken banded individuals are abnormal. The ratio of broken to non-broken is not appreciably different in the two sexes. The broken bands are found not only in bottles exhibbiting the lethal count; they are also found in cultures derived from the lethal strain which do not show a lethal ratio. Individuals known to be heterozygous for the second chromosome recessive involved may have broken bands. The exact relation of the broken bands to this recessive lethal gene is not known; they may be dependent upon other genes. The matter is probably complicated by an environmental effect.

Other abnormalities occurring in the lethal strain with many times the frequency shown by normal stocks include: (1) a slight roughening of the eye, (2) a stunting of one wing or its complete absence; this is usually accompanied by a deformity of the thorax on the same side, (3) a reduction in the size of one or both eyes, and (4) a shrivelling or absence of one leg. Recently these characters, excepting the first, have largely disappeared. It seems probable that they were not definitely correlated with the presence of the lethal gene, but were independent characters present in masked condition in the stock and were brought out by the intensive inbreeding which was practised. It may be noted, however, that abnormalities of this type are common in individuals in which the developmental process is in any way radically upset.

Departures from the normal sex-ratio in *Drosophila melanogaster* are commonly due to an elimination of males by lethal or semi-lethal genes in the X-chromosome. However, sex-ratios exhibiting a deficiency in the number of females produced, although rare, have previously been reported. Subjection to a temperature of 31.5°C. during pupation has been found (MANN 1923) slightly to increase the number of males. Moreover, mutant characters are known, as truncate (ALTENBURG and MULLER 1920), which are somatically somewhat more pronounced in the female than in the male, and which in consequence lead to a somewhat lower relative viability in females. A sex-limited, sex-linked lethal was reported by THOMPSON (abstract, 1921) at the 1920 meeting of the AMERICAN SOCIETY of ZOO-

LOGISTS. This lethal killed all females homozygous for it; males with the lethal were perfectly viable, but held the wings erect. Crosses of heterozygous females with lethal males gave sex-ratios of 1 female : 2 males.

A similar lethal has more recently been described by BONNIER (1923). This lethal kills homozygous females, but has no apparent effect on the males containing it. As in the above case, it gives sex-ratios of 1 female : 2 males. BONNIER has shown how by its means sex-dimorphism can be simulated. He also predicts the finding of autosomal lethals which kill one or the other sex. This prediction is verified by the present lethal.

There is recorded in a recent paper on attached X-chromosomes (L. V. MORGAN 1925) a single culture (No. 184) producing a relatively large number of males. This is from a strain of peculiar behavior (being investigated by T. H. MORGAN) in which the ratio of males to females is variable but high.

Of a different type were the sex-ratios of the unisexual broods reported in 1910 by QUACKENBUSH. One of these cultures produced 135 males and no females, a ratio much like those given by the lethal now reported. It seems reasonable, as has been suggested by STURTEVANT (1920), that the sex-ratios obtained by QUACKENBUSH were due to crosses between *Drosophila simulans* and *Drosophila melanogaster*, which two species were at that time not distinguished (STURTEVANT 1919). The derivation of the parents from mass cultures is suggestive. The sterility of the flies constituting these unisexual broods and their rudimentary gonads, as well as the sex-ratios produced, are all characteristic of crosses of these species, as STURTEVANT'S work shows. In regard to the culture mentioned above which gave a count showing no females, it may be stated that the cross of *Drosophila simulans* female by *Drosophila melanogaster* male, but not the reciprocal cross, gives a greatly lowered proportion of females; the reciprocal cross gives all females.

Striking similarities are shown between a lethal in *Drosophila funebris* reported by MOHR and STURTEVANT (1919) and the present lethal. These investigators found a brood consisting of one female and 87 males. Sex-ratios showing the excess of males were repeated in later generations, but as in the *melanogaster* lethal, there was considerable variation in the sex-ratio, for there was a range from cultures producing no females to cultures in which the sexes were approximately equal in number. Broken abdominal bands were present, but unlike those occurring in *melanogaster* were only rarely found in males. It was believed that those individuals with extreme broken bands did not emerge from the pupa cases. When the strain was crossed to unrelated races the F_1 sex-ratio was 1 female : 1 male, and broken abdominal bands did not occur. Both characters reappeared in F_2. This lethal does not, therefore, show maternal inheritance, and in this respect also it is different from the *melanogaster* mutant.

PROOF OF THE MATERNAL INHERITANCE OF THE LETHAL

In demonstrating the maternal inheritance of the lethal it will be shown that the reciprocal out-crosses produce different results. This, however, is true in out-crosses of ordinary sex-linked mutants. The further demonstration is then necessary that the character reappears for the first time in F_3 rather than in F_2. It will be shown also that the presence in the mother in homozygous condition of a second chromosome gene is necessary in order that the offspring exhibit the lethal count.

It may be stated at the outset that this second chromosome recessive is apparently not the only necessary element involved. This is indicated particularly by the fact that individuals of the same genetic composition with regard to this gene do not always give the same breeding results. That is, cultures sometimes do not give lethal counts which are expected to do so. The converse is not true. The accessory factors are not known; it is possible that environment plays a role.[1] There are indications that modifying or complementary genes exist. This statement is based upon: (1) the relative difficulty with which lethal counts are again obtained once the strain is out-crossed, even when the second chromosome is traced, and (2) the fact that a given female once having given a lethal ratio continues to do so when she is transferred to new bottles. This is strikingly brought out in the work on determining the time of death of the daughters, when it was necessary to make daily transfers of the parents. It has not been considered worth while to attempt a genetic analysis of these modifying genes. Their presence in no way invalidates the conclusion that the lethal effect is maternally inherited and is dependent upon a second-chromosome recessive.

Out-crosses of lethal females

Table 2 shows the immediate results of crosses of females from the lethal strain with males of the stocks indicated. The following genes, in addition to those previously enumerated, are involved. In chromosome II are: star, *S*, 0.0; black, *b*, 46.5; purple, p_r, 52.5; curved, *c*, 73.5 (BRIDGES and MORGAN 1919 and BRIDGES 1921). The curly used is a second chromosome

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[1] Rather extensive attempts to vary the environment by means of acid and temperature have not yielded significant results, chiefly because of the difficulty of breeding this strain under conditions not optimum.

TABLE 2

Crosses exhibiting the lethal count; the female parent in each case is from the lethal strain, the male parent of the type indicated is of different stock.

A. The female parent is known to be virgin.

	P$_1$♂	♀	♂	♂ per 1 ♀
	w^e v f	16	110	6.9
	w^e v f	1	69	69.0
	b pr c	17	98	5.8
	b pr c	1	126	126.0
	b pr c	11	71	6.5
	b pr c	26	106	4.1
	b pr c	1	121	121.0
	b pr c	16	126	7.9
	b pr c	36	128	3.6
	b pr c	0	49	. .
	b pr c	9	46	5.1
	b pr c	0	35	.
	b pr c	38	105	2.8
	b pr c	3	39	13.0
	b pr c	10	53	5.3
	b pr c	1	124	124.0
	b pr c	8	129	16.1
	b pr c	22	50	2.3
	b pr c	6	96	16.0
	b pr c	0	83	.
	b pr c	38	164	4.3
	b pr c	2	162	81.0
	IIIple	18	48	2.7
Total		280	2138	7.6

*B. The female parent is not known to be virgin; for cultures marked * she is known not to be virgin.*

				SHOWING DOMINANT CHARACTERS		
	P$_1$ ♂	♀	♂	♀	♂	♂ per 1 ♀
	S D	2	57	0	28	28.5
	S D	0	90	0	63	. .
	**S D*	18	158	7	82	8.8
	**S D*	38	166	2	13	4.4
	S D	27	65	4	38	2.4
	S D	5	44	1	30	8.8
	Curly	1	56	1	27	56.0
	Curly	5	83	1	14	16.6
Total		96	719	16	295	7.5

stock of the composition $C_{CL}C_{y}c_{n}^{2}C_{CR}/+$. C_y is the gene for curly wings which is generally lethal in homozygous condition, c_n^2 is an allelomorph of cinnabar, and C_{CL} and C_{CR} prevent crossing over (WARD 1923). In this paper "curly" and "non-curly" offspring of the cross of curly stock by the lethal will be mentioned; the former designates offspring containing the C_y-chromosome of the curly stock, the latter may or may not (as will be made clear in the proper place) designate offspring containing the other chromosome. Dichaete, *D*, 40.0, is a third chromosome mutant. The IIIple stock used includes the following third chromosome recessives: roughoid, r_u, 0.0; hairy, *h*, 26.5; scarlet, s_t, 43.8; peach, p^p, 48.0; spineless, s_s, 58.5; sooty, e^s, 70.7 (BRIDGES and MORGAN 1923). It may be further noted that star and dichaete are both dominants and are both lethal when homozygous.

The cross of a female from the lethal strain by an unrelated male may, then, produce the lethal count, and this is true of all the stocks from which males were adequately tested. The lethal counts are not obtained among the F_2 progeny, but may reappear in F_3. The cultures included in table 2B are of particular interest. The female parent of the cultures starred was in each case purposely crossed first to a sib and later to a star-dichaete male. The fact that considerably less than three-fourths of the offspring exhibit one or both of the dominant characters indicates that the female produced offspring by both males. The same is undoubtedly true of the female parent of the last culture (in which cross the expectation is somewhat more curly than non-curly due to the greater relative viability of curly), although she was not intentionally double-mated. In these cases the lethal count is exhibited by the offspring showing dominant characters; moreover after the subtraction of the expected number of normal offspring (assuming that she was mated with the dominant male alone) from the actual number of normal offspring, the remainder also exhibits the lethal count. These crosses show, therefore, that not only may a female from the lethal strain produce a lethal count when mated to a male of unrelated stock, but a single female may be crossed in succession to two different types of male, and the lethal count be shown among the offspring of both males. This is to be expected in the case of a maternally inherited character.

Out-crosses of lethal males

Table 3 shows the results of the cross of males from the lethal strain with females from unrelated stocks. The sex-ratio in F_1 is 1 : 1. The same ratio is shown by the offspring of inbred F_1; it was thought unnecessary to tabulate these results. In the F_3 obtained from these F_2 the lethal counts reappear, as the table shows.

TABLE 3

Crosses not exhibiting the lethal count; the female parent in each case is from the stock indicated, the male parent is from the lethal strain. As shown, offspring of these cultures exhibit the lethal count in the third generation.

	P_1 ♀	♀	♂	♂ per 1 ♀	F_3 lethal counts ♀	♂	♂ per 1 ♀
	S D	64	64	1.0	1	84	84.0
	S D	17	18	1.1	0	42	.
	S D	34	38	1.1	0	75	
		..	..	..	2	54	27.0
	S D	60	52	.9	3	42	14.0
	Curly	49	50	1.0	0	146	
		..	..	.	8	113	14.1
	Curly	10	11	1.1	11	152	13.8
		..			0	131	
		..			0	106	.
	Wild-type	110	104	.9	2	98	49.0
		..			11	51	4.6
	Curly	64	49	.8	26	90	3.5
		.			0	106	
	b pr c	111	117	1.1	17	98	5.8
					1	126	126.0
					11	71	6.5
					26	106	4.1
					1	121	121.0
					16	126	7.9
					36	128	3.6
				.	0	49	
					9	46	5.1
		.		..	0	35	..
			.	.	38	105	2.8
				..	10	53	5.3
			.	.	1	124	124.0
		.	.	..	8	129	16.1
		.	.		22	50	2.3
		.	.	..	6	96	16.0
		.		..	2	162	81.0
	b p_r c	21	28	1.3	3	39	13.0
	IIIple	49	55	1.1	18	48	2.7
	IIIple	47	36	.8	0	83	..
		.	..	...	38	164	4.3
	$\frac{S}{\text{Curly}}\ \frac{D}{+}$	39	37	.9	1	28	28.0
Total		675	659	1–	Total 327	3327	10.2

Reciprocal crosses, then, produce entirely different results in F_1. The disappearance of the lethal ratio in the cross of lethal male by unrelated female is purely temporary, since the peculiarity reappears in F_3.

Crosses from the curly-lethal strain

It was found in crosses between flies from the lethal cultures and flies from curly stock, that when the progeny were inbred, lethal counts might be obtained from non-curly females but they were never obtained from curly females. This is illustrated by tables 4 and 5. The parents of the two sets of cultures shown here differ, so far as known, only as regards one chromosome. For the male parents of both sets are curly and are from the inbred curly-lethal strain; the female parents of table 4 are non-curly and from the curly-lethal strain, the female parents of table 5 are curly from this strain. The former females produce lethal counts, the latter do not. The latter are heterozygous for the second chromosome received from the lethal stock. The former, it is assumed, have become homozygous for this chromosome. Corresponding to the fact that not all non-curly individuals would be expected to be homozygous for this chromosome (since some of them must contain the homologue of the chromosome containing curly in the curly stock), is the fact that not all such non-curly females give the lethal count. That the curly female parents of the cultures of table 5 contained the lethal, although they did not show it, is demonstrated by the fact that their non-curly daughters again produced the lethal counts, as shown. In other words, to produce the lethal count, a

TABLE 4

Crosses exhibiting the lethal count; the parents are from the strain derived by crossing curly sto k with the lethal. The female parent is non-curly; the male parent curly.

	♀	♂	♂ per 1 ♀
	0	131	.
	15	64	4.3
	4	50	12.5
	6	54	9.0
	24	74	3.1
	1	96	96.0
	20	133	6.7
	2	76	38.0
	6	67	11.2
	27	58	2.2
	7	127	18.1
Total	112	930	8.3

female must be homozygous for the second chromosome received from the lethal strain; that is, she must be homozygous for a second chromosome recessive.

TABLE 5

Crosses not giving lethal counts; both parents are curly and are from the strain derived by crossing curly stock with the lethal. From the non-curly daughters lethal counts were again obtained, as indicated.

					Lethal counts from F₁ non-curly ♀ ♀		
	♀	♂	♂ per 1 ♀		♀	♂	♂ per 1 ♀
	84	50	.6		11	152	13.8
	..		..		0	131	..
	..	..	.		0	106	. . .
	91	75	.8		0	146	..
	88	87	1–		26	90	3.5
	..	.	.		0	106	.
	74	70	.9		39	103	2.6
		..	.		30	67	2.2
	105	99	.9		24	83	3.5
	96	102	1.1		12	63	5.3
		.	..		32	91	2.8
	52	62	1.2		9	59	6.6
	58	52	.9		1	96	96.0
	47	60	1.3		21	164	7.8
	43	29	.7		37	113	3.1
	.	..	.		21	103	4.9
	145	162	1.1		10	60	6.0
	119	130	1.1		6	67	11.2
	109	112	1.0		27	58	2 2
	76	74	1–		28	59	2.1
	.	..	..		20	52	2 6
	101	86	.9		2	36	18.0
Total	1288	1250	1–	Total	356	1905	5.4

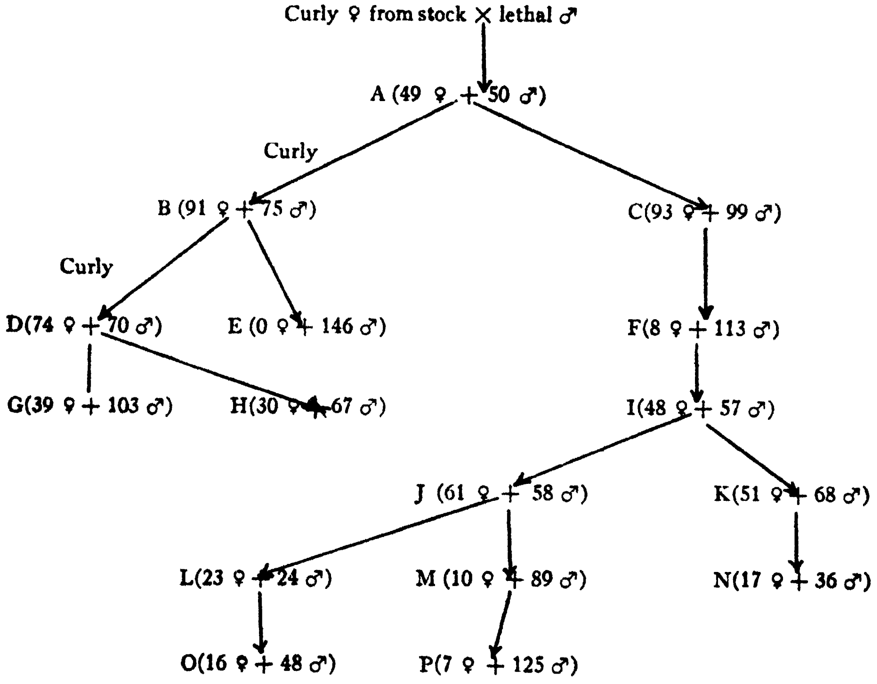
Figure 2 represents a typical pedigree chosen to illustrate the genetic behavior of the lethal. To avoid irrelevant complexity only cultures are included which are in the line of ancestry of lethal counts. As is shown, a lethal male was mated to a female from curly stock, and this cross produced the offspring of culture A which showed a 1 : 1 sex-ratio. Two lines were established from this culture, in one the curly offspring of A were chosen as parents, in the other the non-curly offspring. The mating of curly individuals, B, gave a sex-ratio as expected of 1 female : 1 male. It is to be noted that the only non-curly chromosomes of the second pair contained in this line were necessarily from the lethal strain. Non-curly individuals would be expected to produce lethal cultures. This expectation is verified by the cultures E, G and H.

The second line established, which was from the non-curly offspring of A, arose from the culture C. The parents of this latter culture are heterozygous and are expected not to give a lethal count; C shows a 1 : 1 sex-ratio. But the culture established from offspring of C might involve a mother homozygous for the lethal, correspondingly F does show the lethal count (these are the F_3 of the original cross). Not all of the females of this strain would be expected to give lethal counts, and they do not. All of these results are explicable on the basis of the presence of a second chromosome recessive. All other pedigrees involving the lethal may be interpreted on this same basis, although some of the results indicate the influence of modifying genes, as has been previously explained.

Since it seems unlikely on the basis of the breeding difficulties involved that the mutant will be of particular value in future experiments, the exact locus of the recessive gene in the second linkage group has not been determined. Incomplete data suggest a position near purple (52.5).

FIGURE 2

A typical pedigree showing the genetic behavior of the lethal.



CROSSES WITH A STRAIN WITH ATTACHED X-CHROMOSOMES

Whether the Y of the male, whose sisters die, is responsible *per se* for his survival is a question of interest. If this were true, females containing one extra chromosome, a Y, should be quite as viable as their brothers. To investigate the problem double yellow was used; both sexes of this strain contain a Y chromosome. The X-chromosomes of the female are more or less permanently attached (L. V. MORGAN 1922, 1925) and both contain the recessive gene yellow (*y*, 0.0). A female of this stock, then, obtains her X-chromosomes from her mother alone, she receives also a Y from her father. The male, on the other hand, receives his X from his father and his Y from his mother. It is to be expected that if the lethal is crossed to this double yellow stock, and if the presence of a Y chromosome is responsible for the survival of flies containing it, then succeeding generations should never yield lethal sex-ratios. But in such crosses lethal ratios (accompanied by broken abdominal bands) were obtained, as is shown by table 6; and in the expected proportion of cultures.

TABLE 6

Crosses giving the lethal count, the female parent of each is double yellow.

	Super ♀	♀	♂	♂ per 1♀
	0	43	87	2.0
	0	28	61	2.2
	0	27	67	2.5
	1	31	67	2.2
	0	37	75	2.0
	0	20	50	2.5
	0	43	89	2.1
	1	37	79	2.1
	0	26	55	2.1
	0	4	45	11.3
	0	0	77	
Total	2	296	752	2.5

These results, however, are possibly not to be regarded as significant, for it was learned that a lethal somewhat similar in behavior is present in the attached-X stocks and sporadically gives female deficient ratios. This lethal is being studied by T. H. MORGAN. The lethal counts shown in table 6 are not, therefore, known with certainty to be due to the second-chromosome maternally inherited lethal.[1] Hence they do not prove, as might offhand be concluded, that the Y is not responsible for the greater male viability. The question still remains open.

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[1] Data from the crosses with double yellow have not been included in figures or tables other than table 6.

These crosses with the double yellow stock have bearing, however, on another problem. It might conceivably be held (although in view of considerations later to be presented, this is illogical) that the fact that the female zygotes do not appear is due to their never having been formed. Such an interpretation implies a gametic incompatibility in the lethal strain between an egg from a homozygous mother and the sperm which carries *X*. Now in the strain derived from the cross of lethal by double yellow, the sex resulting from such a combination is male (instead of female as is normally the case). If gametic incompatibility holds, therefore, these crosses will yield male-deficient sex-ratios rather than the female-deficient ratios present in the pure lethal strain. This is not the case; in the crosses involving double yellow no such reversed sex-ratio was obtained. It is believed that this fact probably cannot be attributed in each case to the exact counteracting (or overbalancing) effect of the presence of MORGAN'S lethal. It may be stated, therefore, that this evidence indicates that gametic incompatibility is not responsible for the female-deficient sex-ratio. The conclusion is strengthened by further evidence, as will be shown.

TIME OF DEATH OF FEMALES

On the assumption that gametic incompatibility is responsible for the abnormal sex-ratios it would be expected in the pure lethal strain that practically all eggs from homozygous mothers would be fertilized by Y-containing sperm, since X-containing sperm would be relatively impotent as regards fertilization. The number of males resulting, therefore, would be twice as large as in cultures from the same strain raised under similar conditions and showing non-lethal counts. This apparently is not true.

The fact that the number of offspring in lethal cultures is on the whole approximately half that of non-lethal cultures of the same strain, further shows that the females have not been "transformed" into males. The absence of intersexes tends also to preclude this possibility.

The above reasoning leads to the conclusion, therefore, that the female zygotes are formed but die. Dissection of pupae about ready to hatch shows lethal sex-ratios; death has consequently occurred before this stage.

In order to determine exactly when these zygotes die females were isolated and transferred daily. Each vial in which the females were kept was provided (according to the method of J. C. LI) with a narrow glass slide upon which was placed a slightly smaller strip of dark colored blotting paper impregnated with fermented banana. Eggs were laid on the banana and showed up clearly against this background. The eggs were counted daily; after the elapse of several days the strip was again examined and the number of empty egg cases counted; this number is equivalent to the number of larvae which hatched (empty egg cases are more readily counted than burrowing larvae); a few days later the pupae were counted; as were the adults which emerged.

The conclusions which follow are based upon observations of the development of over 3,500 eggs from females producing lethal counts and of over 2,000 control eggs from a normal strain. Lethal females laid quite as many eggs as the control females. A marked discrepancy occurs, however, in the number of eggs which hatch—only 48 percent of the lethal eggs hatched as opposed to 73 percent of the normal eggs (this percentage is said to be low for normal eggs). Of the larvae from the lethal strain 70 percent pupate, of the larvae from the normal strain 86 percent. Of the pupae from the lethal strain 85 percent hatch into adults; of the normal pupae 95 percent. Thus it is obvious that although the embryo of the lethal strain has a lower viability throughout the entire developmental process, the difference in death rate is much more pronounced in the egg stage than at any other period. Although it cannot be stated definitely that all the females of the lethal strain which do not survive die as eggs, it seems probable that a large majority of these females die at that stage.

DISCUSSION

If a female from the lethal line is crossed to a male from an unrelated stock, the offspring immediately resulting may exhibit the lethal sex-ratio. But if the reciprocal cross is made, the F_1 never exhibit this ratio. Both crosses, alike, give lethal counts in F_3. The immediate offspring of the cross presumably, then, have the same genetic composition (whatever modifiers exist cannot be sex-linked); the only difference being that when the lethal is introduced from the mother most of the daughters die, but when it is introduced from the father they live. In other words, whether a given daughter lives or dies depends not upon her own genetic composition, aside from the fact of her being a female, but upon that of her mother.

We may draw further inferences regarding the nature of this maternal inheritance. It is obvious that the greatest difference between the female zygotes of the reciprocal crosses lies in the origin of their cytoplasm and shell. When the cytoplasm and shell are derived from the lethal line most of the daughters die, but when they are derived from the non-lethal line the daughters live. The cytoplasm or shell (or both) of the eggs from females of the lethal strain has (or have) been so affected that the combination with two X-chromosomes and the autosomes is very much less viable than the combination of non-lethal cytoplasm plus shell with two X-chromosomes and the autosomes, or than lethal (or non-lethal) cytoplasm and shell with X plus Y plus the autosomes. In this connection the reciprocal species crosses of *Drosophila simulans* with *Drosophila melanogaster* (STURTEVANT 1920) may be recalled. As with the present lethal the female offspring of these crosses are rarely able to develop if the egg cytoplasm and shell have been derived from one of the lines, but develop readily if they have been supplied by the other line. That the influence in the case of the lethal now reported is dependent in the final analysis upon the nucleus rather than upon independent cytoplasmic materials is shown by the fact that it is inherited as a second chromosome recessive.

If, in the lethal strain, the chromosomes of a given egg are assumed to be directly responsible for the effect on that egg, it seems probable that the effect becomes established previous to maturation, since, although half of the reduced eggs of a heterozygous female contain the second chromosome recessive, they do not show the lethal effect. However it is not necessarily the case that the chromosomes of the egg itself are responsible for the influence—the effect might conceivably result indirectly from the activity of the maternal chromosomes by means of substances circulating in the blood, or by absorbed materials from the interstitial cells of the ovary, or by means of the egg shell, or by other means. In case the influence is of this indirect type, it may or may not be impressed upon the egg after reduction.

CONCLUSIONS

1. There has been found in *Drosophila melanogaster* a lethal effect the appearance of which is governed by the genetic composition of the mother.
2. The sex-ratio of cultures exhibiting the lethal ratio is variable; the average sex-ratio is 1 female : 5.5 males.
3. A female whose progeny show the lethal sex-ratio must be homozygous for a second chromosome recessive gene.
4. The death of the females which do not appear as adults, probably occurs chiefly in the egg stage. It may be attributed to an influence of the maternal genetic composition upon the eggs which will give rise to the offspring exhibiting the lethal ratio, previous to the time when these eggs leave the mother's body. The presence in such eggs of the female-determining chromosomal complex results in a much higher death rate during development than does the presence of the male-determining chromosomal complex.

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THE MUTANTS OF THE EXTREME LEFT END OF THE SECOND CHROMOSOME OF *DROSOPHILA MELANOGASTER*

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INTRODUCTION

The number of mutant characters known in *Drosophila melanogaster* is continually increasing. Many of the new mutants have no special interest in themselves, but they may serve as tools for the investigation of other problems. Before they can be used with certainty, their general characteristics and linkage relations must be determined.

The important dominant mutant star had been located at the extreme left end of the map of the second chromosome. Four recessive mutants had been found that are very closely linked to star. An account of star, by BRIDGES and MORGAN, has appeared in Carnegie Publication No. 278, pages 259–273. References to the others, namely, telegraph, aristaless, dachsous and expanded, have appeared in several maps and publications, notably "The Genetics of Drosophila," Bibliographica Genetica, 2 : 1–262. The present paper gives, in Part I, descriptions of these mutants and an account of the older data collected by BRIDGES, and, in Part II, the more precise determinations of the locations made recently by STERN. The map based on these determinations is given as figure 1.

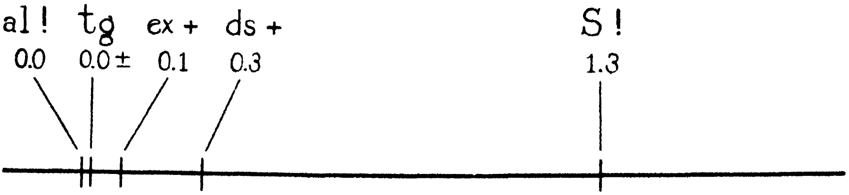

FIGURE 1.—Map of the extreme left end of chromosome II of *Drosophila melanogaster*. This map is based on the data of table 21. The symbol (!) denotes the most useful mutants, (+) those nearly as good, while the absence of a mark indicates that the mutant is important only in special connections.

PART I

Star

The mutant star, found by BRIDGES, February 12, 1915, has been probably the most useful mutant in the second chromosome. This was due primarily to its dominance, its excellent viability (roughly 98 percent that of the wild type) and its very favorable location, since it was the mutant farthest to the left among those whose linkage relations were approximately known. The separation of star from the wild type is not as easy or as rapid as desirable, and hence considerable experience in its use is necessary before the classification is reliable in a critical case.

The character star, as seen under a low power of the binocular (about 12 diameters) differs from the wild type in that the eyes are (1) rougher in

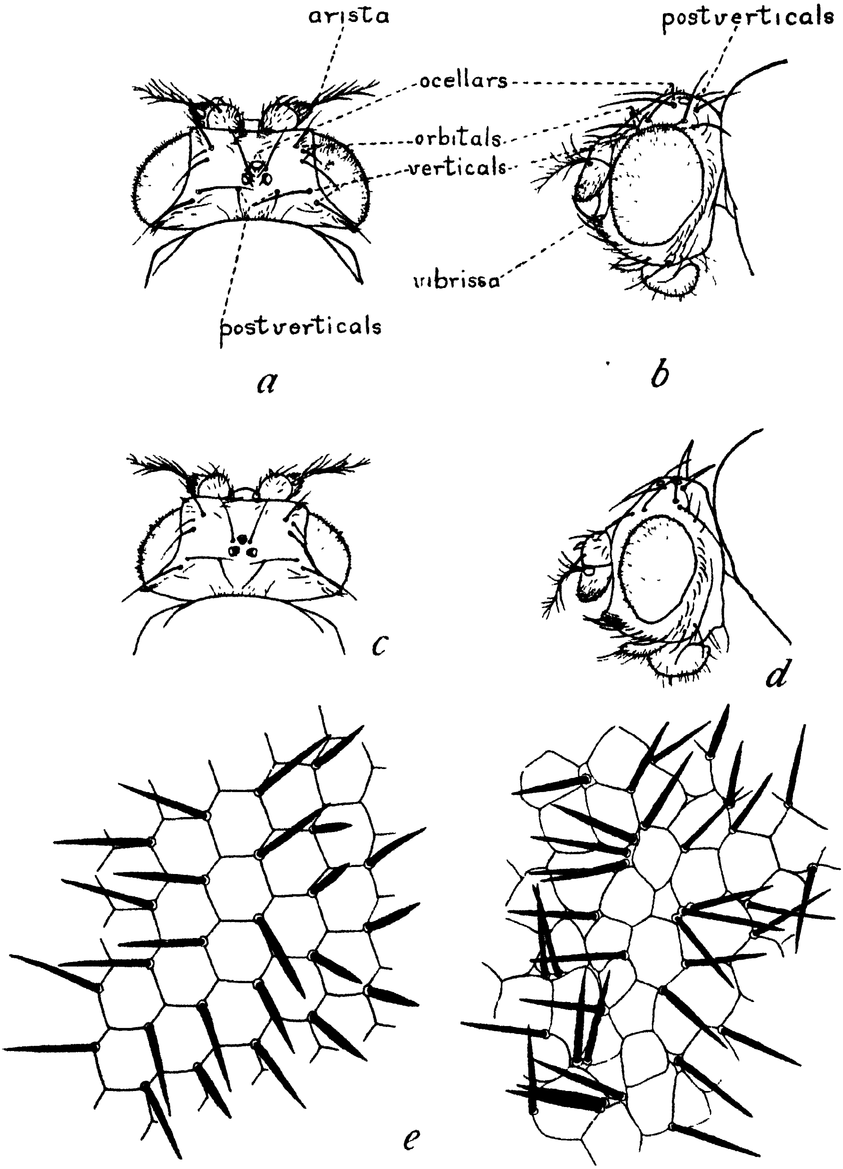

FIGURE 2.—*a* and *b*, top and side views of head of wild-type individual of *Drosophila melanogaster*, with nomenclature of bristles. *c* and *d*, top and side views of the head of the mutant star. *e*, enlarged view of facets and eye-hairs of a wild-type individual (regularity of direction of eye-hairs disturbed by pressure of coverglass). *f*, enlarged view of facets and eye-hairs of the mutant star. (Irregularity in direction of eye-hairs is normal to star.)

texture, (2) somewhat smaller in size, (3) slightly narrower in the antero-posterior direction, (4) slightly darker in color, and (5) faintly scintillating with tiny points of light. (Compare figure 2c with 2a, and 2d with 2b.) A higher power shows that the roughness in texture is due to the ommatidia being arranged irregularly instead of in regular rows, and being of varying size, shape and color (compare figure 2f with 2e). The scintillation is due to reflection of light from the tiny hairs between the facets. In the wild-type eye the hairs stand singly in a perfectly regular lattice-work pattern and are radially directed. In the star eye the hairs are missing from some spaces and aggregated in two's and three's in others, and point in all directions. (Compare figure 2f with 2e.)

The homozygous condition of the star gene is invariably lethal. The character as described is the heterozygous dominant form.

Since the mutant star had more favorable characteristics, especially with respect to its dominance, than the other mutants of the group at the left end of the map, it has been made the point of reference in locating mutants in its neighborhood. For this same reason the data involving star and the mutants throughout the remainder of the chromosome are greater in amount and better in quality than the data in which the other leftmost mutants are involved. This difference will probably continue for some years and accordingly the locus of star will maintain its prime importance in the region to the left of black.

Reappearances of star

Star, or an allelomorph so similar as not to be distinguished by inspection or in the course of experimentation, has reappeared by mutation on four occasions:

(1) In a cross between two stocks involving only sex-linked characters (cut female by eosin miniature garnet^2 male) BRIDGES found that half the offspring in one pair culture showed a star-like eye (culture 8718, June 8, 1918). Examination of the original stocks showed no star-like individuals present, nor did the pedigrees of those stocks involve star. Tests showed that the new character was a heterozygous dominant, lethal when homozygous, located in chromosome II, and much to the left of black. Crosses between the new star and the original gave in F_1 the typical 2 star : 1 wild type ratio (8756,8757; total 262 star : 132 wild type).

(2) MOHR found star again (July 20, 1920) and has reported the facts for this case (MOHR 1923).

(3) The third reappearance of star occurred in the spring of 1925 in the cultures of L. V. MORGAN. Allelomorphism was established by

the fact that a cross between a stock of the new star balanced by curly (Ward 1922) and a stock of the original star balanced by curly gave in F_1 only flies that were both star and curly, exactly as though either star had been bred to itself in balanced stock. (Unpublished data of L. V. Morgan.)

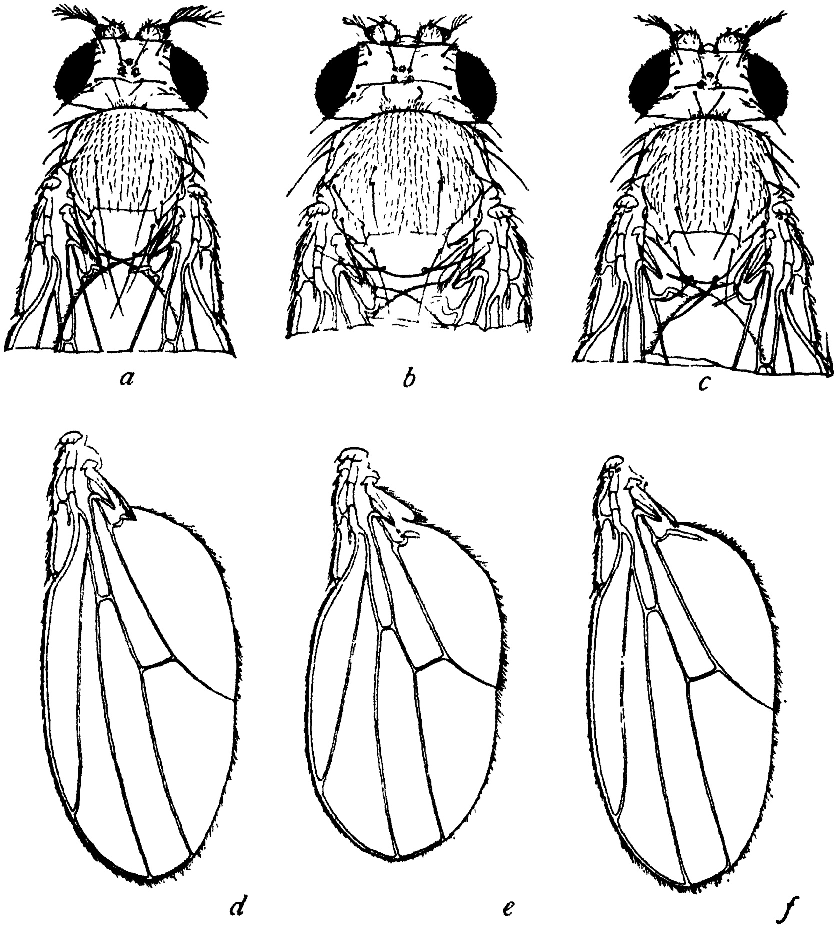

Figure 3.—*a* and *d*, dorsal view of head and thorax and a wing of a wild-type individual of *Drosophila melanogaster*. *b*, the mutant aristaless, showing reduced aristae and posterior bristles on the scutellum widely separated and strongly divergent. *c*, the mutant telegraph, showing the slight erection of the posterior bristles of the scutellum. *f*, the wing of the mutant telegraph, showing weak spots in the second longitudinal vein. *e*, the wing of the mutant dachsous, showing the crossveins close together.

(4) In a cross involving the second-chromosome dominant curly Doctor Helen Redfield found one curly female with an eye like star.

The character was found to be dominant and due to a gene in the curly-bearing second chromosome. In crosses to the original star it was found that the new star was lethal in the compounds with the old star, and hence must be an allelomorph of star. (Unpublished data of Doctor H. REDFIELD.)

Telegraph

In establishing a pure stock of the third-chromosome recessive smudge, BRIDGES found a culture (No. 3947, March 27, 1916), in which a majority of the flies showed a break or weak space in the second longitudinal vein (Compare figure 3f with 3d). Many of the flies showed more than one such break, and from this dot-and-dash appearance of the vein the mutant was called telegraph. When telegraph males and females were bred together most of the progeny showed the character. This indicated that the mutant was a recessive for which the stock was homozygous but that the character overlapped the wild type. In some individuals telegraph failed to show, and in others could only be distinguished with difficulty in one wing. Later it was found that many of the telegraph flies showed another characteristic, namely, the posterior scutellar bristles were more or less erect, as in vestigial, instead of pointing backward. (Compare figure 3c with 3a.) This bristle characteristic could often be used as an index of the mutant in those flies in which the breaks were absent or weak.

Telegraph by star backcross

Telegraph males were crossed to star females, and in F_1 the telegraph character was found to be recessive. The F_1 star flies were inbred in pairs for an F_2 generation (table 1). The telegraph character reappeared in slightly less than the expected frequency. Only four of the F_2 flies showed both star and telegraph, while 271 showed simply telegraph. This strong linkage showed that the locus of telegraph is in the second chromosome, and also that it is very close to that of star. Only two flies that were not-

TABLE 1

P_1, telegraph ♂ × star ♀; F_1 star ♀ × F_1 star ♂.

MAY, 1917	TELEGRAPH	STAR	TELEGRAPH STAR	WILD TYPE
7203	86	220	1	1
7204	80	194	3	1
7205	105	210	..	..
Total	271	624	4	2

star failed to show telegraph. These were probably due to crossing over rather than to overlapping of the telegraph character. The percentage of recombination was calculated from the average of the two recombination classes, as compared with the original combination class telegraph

$$\left(\frac{3}{271+3}\times 100=1.1\right)$$

The star class could not be used in the calculation since it was complex, including recombinations as well as original combinations.

From the star class of table 1 virgin females were mated to telegraph males for backcross counts (table 2). Some difficulty in classification was encountered, and the class marked wild type (+) was composed partly of true recombinations due to crossing over but mostly of overlaps from the genotypically telegraph class. The percentage of recombination was at first calculated from the total flies of tables 1 and 2 as 2.0, and in the maps prepared (see especially the maps in BRIDGES 1921 and in MORGAN, BRIDGES and STURTEVANT 1925) the telegraph star distance was given as 2.0. Because the phenotypic class wild type was partly due to overlaps the calculations should have been made on the basis of the telegraph flies alone, and would then have been 0.7

TABLE 2

Telegraph over star ♀ (*from table 1*)×*telegraph* ♂.

MAY, 1917	TELEGRAPH	STAR	TELEGRAPH STAR	WILD TYPE
7249	107	105	2	2
7250	63	63	1	1
7251	49	49		5
7252	215	208	1	4
7268	92	89		4
CONTINUED				
7323	29	35	1	2
7648	161	134		7
7649	142	161		8
7654	78	79		2
Total	936	923	5	35

$$\left(\frac{9}{1216}\times 100=0.7\right)$$

Telegraph black stock

A female showing both telegraph and star (from table 2) was crossed to a black male. F_1 heterozygous star females were crossed to telegraph males (culture 7383, telegraph star = 71, wild type = 79, telegraph = 2, star = 1). Both of the recombinational telegraph males that occurred were crossed to black of unrelated stock. Half of the offspring were black. These black flies were inbred and in the next generation somewhat less than a quarter of the flies showed telegraph as well as black. These latter were inbred to give a telegraph black stock. The fact that the above procedure was successful in giving a telegraph black stock made it seem probable that the locus of telegraph is to the left of star rather than to the right. If it were to the right, the telegraph flies tested must have resulted from the less frequent double crossing over. A three-point backcross involving telegraph star and black was undertaken to test this point, but difficulties in classification prevented it from being conclusive.

As will be shown in a later section of this paper, the work of STERN has shown that the locus of telegraph is indeed to the left of that of star, and that the distance between them is about 1.3 units.

Aristaless

In examining an F_2 culture (8028, November 7, 1917) involving eosin, vermilion, forked and a new mutant "disbanded" that had arisen in the California wild stock, BRIDGES found that approximately a quarter of the flies showed posterior scutellar bristles that were erect, diverged strongly, and were widely separated from each other (Compare figure 3b with 3a). A pure stock of this recessive was established and freed of the other mutant characters originally present. It was then found that the flies that showed "repelled" bristles were further characterized by great reduction or even complete absence of the aristae (figure 3b). It was anticipated that this mutant, called aristaless, would be especially valuable since it affected a region of the body affected by few other mutants and hence could be used widely without confusion in classification.

Aristaless by star backcross

Aristaless was crossed to star dichaete, and several F_2 cultures were raised. Aristaless occurred freely in the dichaete F_2 flies but apparently not at all in the star F_2 flies. From this it was concluded that the locus of aristaless is in the second chromosome, and also that it is very close to that of star.

From the F_2 flies, star females and aristaless males were bred together for backcross counts (table 3). In two of the cultures the viability of aristaless was very low, but in the other two it was normal. There were three recombinations in a total of 334 flies, or 0.9 percent of recombination.

Since at that time work on the third-chromosome mutants was being carried on practically to the exclusion of other work, as soon as the above data were obtained the mutant was turned into stock without further testing. In making the earlier maps of the second chromosome the locus was put down as to the right of star, since the probability that any mutant should lie to the left of star was thought to be less than that it should be to the right. The recent tests of STERN (see below) have shown that the locus of aristaless is in reality to the left of that of star, and that the distance between them is roughly 1.0 units.

TABLE 3

F_2 *aristaless over star* ♀ × F_2 *aristaless* ♂.

DECEMBER, 1918	ARISTALESS	STAR	ARISTALESS STAR	WILD TYPE
9029	10	47	..	
9187	19	59	1	2
9198	43	43	..	..
9263	50	60	..	..
Total	122	209	1	2

Aristaless^2

In looking over the second-chromosome stock "fringed," STERN found several flies of both sexes with reduced aristae and erected posterior scutellar bristles (January 1926). Fringed was kept as a balanced stock over curly. The fact that all flies exhibiting the new mutant were fringed not-curly indicated that its locus was in the not-curly second-chromosome. This, together with its characteristics, made it probable that one was dealing with an allelomorph of aristaless. By crossing the mutant flies to aristaless and obtaining only aristaless-like flies in the F_1 generation, the allelomorphism was proven. Aristaless^2 is a somewhat less extreme character than the old aristaless, both in regard to the reduction of the aristae and the erection of the scutellar bristles. The classification is as easy as in aristaless. If viability tests should show a better viability of aristaless^2 than of aristaless, then the former would replace the old allelomorph in future work.

Dachsous

In a mixed stock of black and black purple (Culture 9068, November 12, 1917), BRIDGES noticed that a small percentage of the flies had wings which were shorter than normal and in which the crossveins were very close together (figure 3e). The abdomen and legs were also shorter, and the general appearance of the fly was similar to that of the mutant dachs. The "dachsous" flies were inbred, and they produced only dachsous offspring, which showed that the character was a recessive. A dachsous black stock and a dachsous black purple stock were isolated.

Dachsous black backcross

Three cultures of a dachsous black backcross were raised (table 4). They gave respectively 22, 25 and 27 percent of recombination for dachsous and black. This linkage ratio showed that the locus of dachsous is in the second chromosome.

TABLE 4

P_1, dachsous black×wild type; BC., F_1 wild type ♀×dachsous black ♂.

OCTOBER, 1920	DACHSOUS BLACK	WILD TYPE	DACHSOUS	BLACK
12055	104	118	26	36
12056	86	85	26	32
12061	116	118	40	45
Total	306	321	92	113

Dachsous black purple by lobe^2 backcross

Dachsous black purple males were crossed to females with the dominant character lobe^2, whose locus is at 70 in the second chromosome (BRIDGES 1921). In the backcross cultures (table 5) the percentage of recombination for dachsous and black was 36.4, for dachsous and purple 38.9 and for dachsous and lobe^2 41.0. That is, the locus of dachsous is farthest from that of lobe and hence is to the left of black. When the locus of dachsous was first calculated on the basis of the data of tables 4 and 5, proper allowance was not made for double crossing over and the locus was entered as probably 12 units to the right of star (see MORGAN, BRIDGES and STURTEVANT 1925, page 92).

TABLE 5

P_1, dachsous black purple ♂×lobe^2 ♀; BC., F_1 lobe^2 ♀×dachsous black purple ♂.

NOVEMBER 1920	DACHSOUS BLACK PURPLE	LOBE^2	DACHSOUS LOBE^2	BLACK PURPLE	DACHSOUS BLACK LOBE^2	PURPLE	DACHSOUS BLACK PURPLE LOBE^2	WILD TYPE	DACHSOUS PURPLE	BLACK LOBE^2	DACHSOUS	BLACK PURPLE LOBE^2	DACHSOUS BLACK	PURPLE LOBE^2	DACHSOUS PURPLE LOBE^2	BLACK
12147	98	98	71	49	5	7	21	13	1		9	9	3	..		
12154	104	115	54	71	4	4	18	12	.		11	9		1	1	
12155	75	114	53	51	4	1	15	14	1	..	5	10	1	3	..	2
12256	95	100	59	59	10	3	17	18	1	2	14	15	3	1		
12157	72	92	42	49	3	3	9	10	4		7	9	..	2	.	.
Total	444	519	279	279	26	18	80	67	7	2	46	52	7	7	1	2

Dachsous was given to MOHR for use in studying the relationships of the supposed deficiency "gull," and MOHR reported by letter that he found that dachsous and star gave about 1 percent of recombination. The tests of STERN (see below) have shown that the locus of dachsous is to the left of star by about 1.0 unit.

Dachsous^2

The stock of speck was found by BRIDGES to be homozygous for a character that resembled dachsous, (April 2, 1925). Crosses between this mutant and dachsous gave only flies like dachsous. In dachsous^2 the crossveins are as close together as in dachsous, but the wings, legs, and body are only slightly shortened.

Dachsous^3

In a stock of wild flies collected by Doctor D. E. LANCEFIELD at Olympia, Washington, in the summer of 1925, BRIDGES found several males and females that had crossveins very close together (November 5, 1925). Such flies reappeared continuously in several generations, usually late in the course of the culture. Attempts to breed them together failed. Likewise females used in outcrosses produced no offspring. Four such females, that were fully ten days old, were dissected and it was found that two of them contained no ovaries. The oviducts were present and a small tuft of trachae where the ovaries are normally situated. One female differed in that one partly developed egg was present. The fourth had five such eggs. Of these, four stuck out radially from the tuft of trachae and broke away easily. The other egg was partly within the oviduct, wrong end foremost. Of approximately twenty females used in crosses none gave offspring. The males proved fertile and a self-perpetuating stock was obtained by crossing them to curly, and then inbreeding the curly F_1 flies. The fact that the F_2 flies in this stock were all of two classes, simple curly and those with close-crossveins but not-curly, showed that the locus of the mutant is in the second chromosome.

Dachsous^3 by star backcross

A backcross with star gave less than one percent of recombination (table 6). Crosses of close-crossvein to dachsous gave only flies with close-crossveins. Accordingly the new mutant is an allelomorph of dachsous and is called dachsous^3. The compound between the two allelomorphs dachsous and dachsous^3 had wings nearly as long as dachsous^3, abdomen occasionally shortened but not so strikingly as in dachsous. The viability and fertility of the compound were apparently normal.

In viability dachsous^3 was very poor, 50 percent that of star in table 6, while dachsous has always been excellent (99 percent that of wild type).

TABLE 6

P_1, *dachsous*^{3}×*star*; *BC.*, F_1 *star* ♀×*dachsous*^{3} ♂.

DECEMBER 1925	DACHSOUS^3	STAR	DACHSOUS^3 STAR	WILD TYPE
15233	53	96	. .	1
15236	77	167	1	1
Total	130	263	1	2

Dachsous^3 was delayed in emergence; few dachsous^3 flies appeared until the not-dachsous^3 flies had been hatching for five days. The first dachsous^3 flies to emerge had no bristles on the scutellum, or around the ocelli, and the scutellum was shortened. The wings were spread slightly and often lacked the anterior crossveins. The flies that hatched later had normal head and scutellum and there were even one or two extra bristles on the scutellum. The wings and abdomen of dachsous^3 were not shortened as much as those of dachsous or dachsous^2.

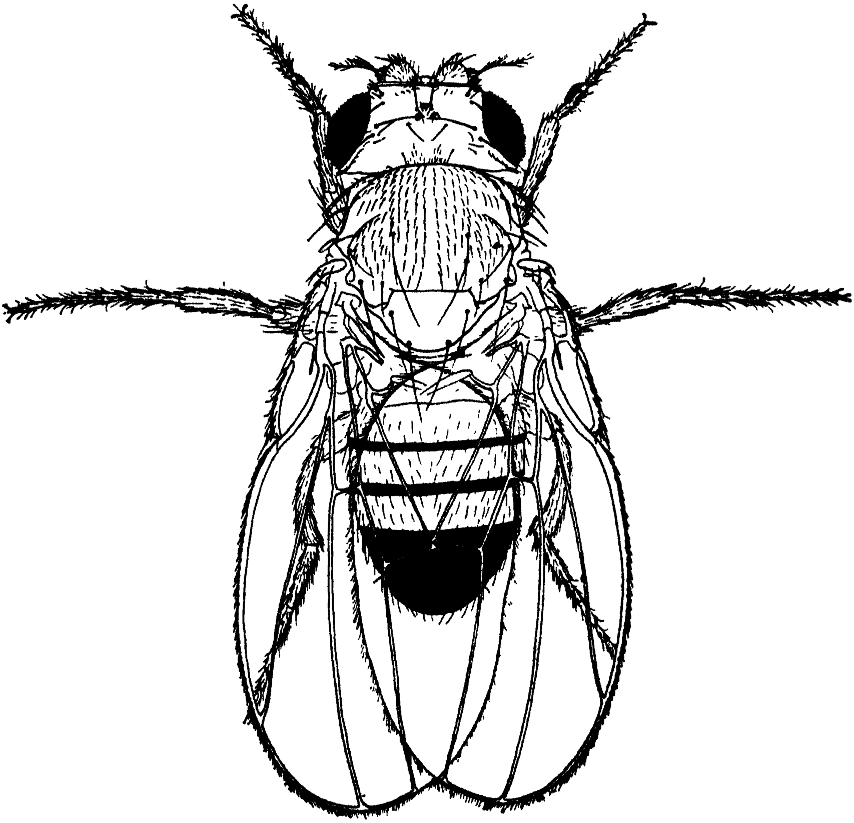

FIGURE 4.—The mutant expanded, showing large, broad wings.

Expanded

In determining the locus of the sex-linked lethal, lethal 15, **Bridges** found that one culture (8118, November 21, 1917), was giving a few flies with wings very broad, and similar in posture to curved, namely, extended rather widely at the sides and held above the back, except that a curvature of the wing brought the tips of the wings down to the normal level (figure 4). The crossveins were also closer together than normal. The eyes were somewhat reduced and roughish in texture. The flies were larger than normal.

Expanded by star backcross

"Expanded" males were out-crossed to star dichaete females, and in F_1 gave only not-expanded progeny, from which the character was known to be recessive. F_1 star dichaete females were crossed to expanded males that had been secured by breeding together expanded males and females from the original culture (table 7).

Table 7

P_1, *expanded* ♂×*star* (*D*) ♀; *BC*., F_1 *star* (*D*) ♀×*expanded* ♂.

November 1917	Expanded	Star	Expanded star	Wild type
8250	70	80	3	9
8295	44	52	..	8
8365	219	237	2	4
8391	150	157	16	2
8393	134	149	6	4
Total	617	675	27	27

The different cultures gave contradictory results. The individuals classified as recombinations were probably mostly errors due to unfamiliarity with the character and to confusion between the roughish eye of expanded, and the roughish eye of star. The apparent percentage of recombination was 4.0, and accordingly the locus of expanded was set down tentatively as about 4 units to the right of star.

Stern (see below) found that the reduction in the eye of expanded tends to increase as the cultures become older; while on the other hand the wing character becomes less extreme in older cultures. Trustworthy classifications could be made for expanded and star after familiarity with the interaction of the two had been acquired. The locus was found by **Stern** to be about 1.2 units distant from star, and to be to the left, not to the right.

PART II

Preliminary tests of BRIDGES, reported in Part I, had shown that the dominant character star and the four recessive characters telegraph, aristaless, expanded, and dachsous form a group of genes at the left end of the map of the second chromosome. There still remained to be determined, first, the sequence of the loci of these genes within the group, this being the most important and constant feature represented by a chromosome map, and second, the relative distances between the loci.

The first step in the procedure was to find more exactly, by a series of two-point backcross experiments, on a fairly large scale, the distance of each of the recessives from the locus of star, which, on account of its dominance, was the easiest point of reference.

Thus, the general procedure in the first series of tests was to cross star to a given recessive, and then to mate several of the F_1 star double heterozygous females

$$\frac{r \qquad +^{s}}{+^{r} \qquad S}$$

each to a male of the recessive stock. Since star is a dominant, the male of the recessive stock is in effect a double recessive

$$\frac{r \qquad +^{s}}{r \qquad +^{s}}.$$

The results of the series of distance tests should eliminate certain sequences of loci as improbable and limit the number of subsequent tests.

Telegraph by star backcross

On inspection of the telegraph stock it was seen that about half of the flies failed to show any break in the longitudinal vein. And in the telegraph by star backcross results this same overlap was observed. In calculating recombination percentages for cases in which one of the mutant characters overlaps wild type, fairly correct values may be obtained by disregarding all flies that fail to show the character in question and using only those flies in which the character was seen. This method was followed in Part I in calculating the telegraph star recombination percent.

However, in the F_1 of the telegraph by star cross it was seen that about 6 percent of the flies heterozygous for telegraph showed breaks in the longitudinal veins. In the backcross results the flies in which the breaks were due to dominance of the character could not be distinguished from the flies in which the breaks were the homozygous recessive form. Consequently the calculations could not be made on the basis of the flies that showed the break. It was found that the other characteristic of the telegraph mutant, namely, erected scutellar bristles, was strictly recessive and accordingly in making the classifications telegraph flies were identified solely by use of this index. The erect-bristle character also overlapped the wild type by about 50 percent and in making the calculations all not-erected flies have been disregarded. The fifteen cultures (table 8) produced a total of 1169 flies with erected bristles, of which 11, or 0.9 percent were star and were therefore recombinations due to crossing over between the loci of telegraph and star.

TABLE 8

P_1, *telegraph* ♀ × *star* ♂; *BC.*, F_1 *star* ♀ × *telegraph* ♂ (*Jan. 1925*).

TELEGRAPH	TELEGRAPH STAR	TELEGRAPH	TELEGRAPH STAR	TELEGRAPH	TELEGRAPH STAR	TELEGRAPH	TELEGRAPH STAR
56	.	95	.	98	1	43	1
49		70	1	56	. .	99	. .
41	1	70	1	97		134	3
85	1	68	2	97			
						1158	11

Aristaless by star backcross

The character aristaless, like telegraph, has two prominant characteristics, namely, the aristae are reduced or even absent, and the posterior scutellar bristles are erected, strongly divergent and situated abnormally far apart. It was found that the arista character, though fluctuating in the amount of reduction, is a safe index of the homozygous recessive character. But the scutellar bristles character was occasionally

TABLE 9

P_1, *aristaless* ♀ × *star* ♂, *BC.*, F_1 *star* ♀ × *aristaless* ♂. (*Jan. 1925*).

ARISTALESS	STAR	ARISTALESS STAR	WILD TYPE	ARISTALESS	STAR	ARISTALESS STAR	WILD TYPE
118	131	.	1	64	67	1	.
61	57	3	2	131	173	3	1
73	87	1	1	149	157	4	1
69	119	. .	.	61	72	. .	1
120	120	2	1	99	108	1	1
115	126	4	1	124	136	3	2
85	110	1	2	18	32	1	1
148	154	2	1	37	34	2	. .
166	168	1	4	188	176	1	2
53	64	2	2				
156	162	1	1	2035	2244	33	25

apparent in heterozygous flies, and in homozygous flies was sometimes difficult to distinguish. The classification throughout has been on the basis of the arista characteristic of "aristaless."

Two sets of backcross cultures (table 9; 1, the first seven cultures and 2, the remainder) were raised, and these agreed in the percentages of recombination (1.4 and 1.3). The total number of recombinations in the two sets was 58 in a total of 4337 flies, or 1.3 percent.

Dachsous by star backcross

The new dachsous by star backcross (table 10) gave a total of 4269 flies of which 31, or 0.7 percent, were recombinations.

TABLE 10

P_1, dachsous ♀ × star ♂; BC., F_1 star ♀ × dachsous ♂. (Jan. 1925).

		DACHSOUS				DACHSOUS	
DACHSOUS	STAR	STAR	WILD TYPE	DACHSOUS	STAR	STAR	WILD TYPE
173	175	1	4	170	166	2	2
159	174	..	1	190	205	.	2
96	123	1	3	225	209	2	3
156	163	1	1	203	219	1	1
193	176	..	1	130	161	2	.
157	144	..	1				
235	236	1	1	2087	2151	11	20

Expanded by star backcross

The new expanded by star backcross (table 11) gave a total of 3060 flies, of which 46, or 1.5 percent, were recombinations.

TABLE 11

P_1, expanded ♀ × star ♂; BC., F_1 star ♀ × expanded ♂. (Jan. 1925).

		EXPANDED				EXPANDED	
EXPANDED	STAR	STAR	WILD TYPE	EXPANDED	STAR	STAR	WILD TYPE
149	118	1	3	190	179	4	1
153	138	6	2	297	238	5	5
169	169	2	1	206	204	2	1
162	173	2	5	148	191	2	2
68	62	1	1				
				1542	1472	25	21

The results of the above tests with star confirmed the earlier finding of BRIDGES that the loci of the five mutants lie very close together (table 12). The loci were found to be even closer together than expected. No certain conclusion as to the relative distances could be drawn, since the probable errors of such small percentages are high, and the possible effects of linkage modifications in the different experiments could not be excluded.

TABLE 12

Summary of two-point data of Part II.

LOCI	TOTAL	PERCENT RECOM.
Aristaless-star	4337	1.3
Telegraph-star	1169	0.9
Expanded-star	3060	1.5
Dachsous-star	4269	0.7

The next step was to build double recessive stocks of the recessive mutants. By use of such stocks, together with star, a series of three-point experiments could be made that would give decisive evidence as to the sequence of the three loci of each experiment and also relative distances found under the same condition with respect to crossover modifiers. In the process of building the double recessives, evidence could be obtained as to the relative order of certain of the genes.

Telegraph expanded double recessive

The first double recessive sought was telegraph expanded. From the telegraph by star backcross of table 8 recombinational telegraph star flies were obtained which were mated to expanded. The F_1 star females were then crossed to telegraph males. If expanded were on the opposite side of star from telegraph

$$\begin{matrix} t_g & S & + \\ + & + & e_x \end{matrix}$$

as seemed probable from the preliminary results of BRIDGES, then the telegraph not-star flies which occurred in the offspring of this experiment as a result of crossing over between telegraph and star should have the constitution

$$\frac{t_g \quad e_x}{t_g \quad +}.$$

By breeding together such recombinational telegraph flies, the double recessive telegraph expanded should be obtained as about 25 percent of the telegraph offspring. But when this mating was made it was found that none of the offspring were expanded. This showed that the assumed order was incorrect, and that, in fact, the loci of telegraph and expanded are both on the same side of star. The loci of telegraph and expanded must therefore be very close to each other, but there was no evidence as to which of the two is further from star. On the supposition that the order is expanded telegraph, an expanded star was crossed to telegraph, and the F_1 star female was backcrossed to expanded (table 13). The F_2 expanded flies were crossed to telegraph, but none gave telegraph offspring.

TABLE 13

P_1, expanded star×telegraph; BC., F_1 star ♀×expanded ♂. (July 1925).

EXPANDED STAR	EXPANDED	EXPANDED STAR	EXPANDED	EXPANDED STAR	EXPANDED	EXPANDED STAR	EXPANDED
99	1	43	1	63	2	73	..
156	.	89	..	93	2	40	1
142	1	49	.	79	1	70	.
96	..	62	.	77	3	88	1
80	1	66	1	90	..	93	1
35	.	87	.	79	1	56	..
30	1	86	2	72	..		
						2143	21

Similarly, on the supposition that the order is telegraph expanded, a telegraph star fly was crossed to expanded, and the F_1 star females were backcrossed to telegraph males (table 14). The F_2 telegraph not-star flies that resulted from crossing over were crossed to expanded. In the off-spring of this cross (F_3) expanded flies were produced, which were inbred and which gave telegraph expanded in the next generation (F_4). The order is then established as telegraph, expanded, star (or star, expanded, telegraph). The stock of telegraph expanded was lost in traveling, and the three-point experiment could not therefore be carried out.

TABLE 14

P_1, telegraph star×expanded; BC., F_1 star ♀×telegraph ♂. (July 1925).

TELEGRAPH STAR	TELEGRAPH	TELEGRAPH STAR	TELEGRAPH	TELEGRAPH STAR	TELEGRAPH	TELEGRAPH STAR	TELEGRAPH
82	..	37	..	74	1	59	..
82	1	52	2	31	..	87	
106	.	34	..	34	..	35	
62	1	35	.	35	..	26	.
38	.	51	1	84	2	37	
14		33	3	51	..	62	1
37		23	..	82	4	49	..
30	2	45	.	67	4	39	
30	..	90	3	77	.		
						1810	25

Telegraph dachsous double recessive

Telegraph flies were also crossed to dachsous, and the F_1 star females were backcrossed to telegraph males (table 15). The F_2 recombinational telegraph not-star flies were bred together, and in the next generation telegraph dachsous flies were obtained. This fact shows that dachsous is either on the opposite side from telegraph

TABLE 15

P_1, telegraph star × dachsous; BC., F_1 star ♀ × telegraph ♂. (Mar. 1925).

TELEGRAPH STAR	TELEGRAPH	TELEGRAPH STAR	TELEGRAPH	TELEGRAPH STAR	TELEGRAPH
74	..	51	3	72	1
83		56	1	65	1
65	.	79	1		
				545	7

$$\frac{t_g \qquad + \qquad d_s}{+ \qquad S \qquad +},$$

or, if telegraph and dachsous are on the same side of star, that dachsous is nearer to star than is telegraph

$$\frac{t_g \qquad + \qquad S}{+ \qquad d_s \qquad +}.$$

It had been supposed that the telegraph dachsous double recessive might be too difficult to distinguish from simple dachsous, since dachsous itself causes a slight erection of the scutellar bristles. However, it was found that often the bristles of the double form were extremely erected, even directed forward, and that telegraph could be identified in dachsous flies in about the same percentage (50±) as simple telegraph could be distinguished from wild type.

Telegraph star by dachsous backcross

The three-point backcross involving telegraph, dachsous and star (table 16) showed that telegraph and dachsous are on the same side of star, since the crossing over between them was much less frequent than between either and star. The telegraph dachsous recombination percentage was 0.1, while the telegraph star recombination percentage was 1.2 and the dachsous star 1.1. The telegraph star percentage was greater than the dachsous star percentage, which agrees with the order telegraph, dachsous, star. In a three-point backcross the class due to double crossing

TABLE 16

P_1, telegraph star×dachsous; BC., F_1 star ♀×telegraph dachsous ♂. (April 1925).

TELEGRAPH STAR	TELEGRAPH DACHSOUS	TELEGRAPH	TELEGRAPH STAR	TELEGRAPH DACHSOUS	TELEGRAPH	TELEGRAPH STAR	TELEGRAPH DACHSOUS	TELEGRAPH
75	1	2	92	..	1	53	..	..
88	..	..	62	..	2	73	..	1
58	..	..	92	..	1	88	..	1
75	..	..	73	..	2	64	..	1
71	1	1	81	..	..	114	..	1
69	..	1	61	..	..			
58	..	2	88	..	..	1435	2	16

over is the smallest class, and where the loci are very close together, as in the present case, it is not expected to be realized at all. The absence of flies of the telegraph dachsous star class shows that this was the double crossover class, and that the orientation of the genes was

$$\frac{t_g \quad + \quad S}{+ \quad d_s \quad +}.$$

Aristaless dachsous double recessive

A dachsous star fly (from table 10) was crossed to aristaless and the F_1 star females were crossed to dachsous. The dachsous not-star flies that occurred as the result of crossing over were inbred. None of their progeny were aristaless. From this it was evident that the locus of aristaless is not on the opposite side of star from dachsous

$$\frac{d_s \quad S \quad +}{+ \quad + \quad a_l}$$

but that it is on the same side. A second method consisted in crossing an aristaless star (from table 9) to dachsous and again mating the F_1 star females to aristaless males. The recombinational aristaless not-star flies were inbred, and in some cases there were produced the double recessive aristaless dachsous. The production of the double from the cross showed that the orientation of the genes was

$$\frac{a_l \quad + \quad S}{+ \quad d_s \quad +}.$$

Aristaless star by dachsous backcross

The three-point backcross experiment (table 17) confirmed this order, since the double crossover classes, not realized, were aristaless dachsous star and wild type, and the orientation of the genes was accordingly

$$\frac{a_l \quad + \quad S}{+ \quad d_s \quad +}.$$

TABLE 17

P_1, aristaless star×dachsous; BC., F_1 star ♀ ×aristaless dachsous ♂. (April 1925).

ARISTALESS STAR	DACHSOUS	ARISTALESS DACHSOUS	STAR	ARISTALESS	DACHSOUS STAR	ARISTALESS STAR	DACHSOUS	ARISTALESS DACHSOUS	STAR	ARISTALESS	DACHSOUS STAR
130	153	..	1	1	1	116	165	..	1	2	2
106	170	1	..	.	2	159	204	..	1	1	1
139	158	..	..	1	2	145	190	1	1	1	1
148	155	2	..	..	2	76	77	..	3	..	1
65	91	..	..	..	..	169	213	..	..	1	2
67	112	..	..	1	2	158	184	..	..	1	2
91	139	1	..	1	1						
137	149	..	..	2	3	1706	2160	5	7	12	22

The recombination percentages from table 14 were aristaless dachsous 0.3, aristaless star 1.2, and dachsous star 0.9 percent.

Aristaless expanded double recessive

An expanded star fly, from table 11, was crossed to aristaless, and the F_1 star flies were backcrossed to expanded. The expanded not-star flies that occurred were then crossed to aristaless. None of the offspring of this later type of cross showed aristaless. From this result it was clear that aristaless and expanded lie on the same side of star; for if the orientation had been

$$\frac{e_x \quad S \quad +}{+ \quad + \quad a_1} \text{ or } \frac{a_1 \quad + \quad +}{+ \quad S \quad e_x}$$

then all the recombinational expanded flies would have produced aristaless.

The two-point data summarized in table 12, showed that the loci aristaless and expanded must be very close indeed to each other since the expanded star recombination percent was 1.5, and the aristaless star percent 1.3, which is very little different.

Aristaless star by expanded backcross

A second experiment was started by mating a female that carried aristaless and star in one chromosome and expanded in the other to aristaless males. Twenty-nine of the recombinational aristaless not-star flies were mated to expanded, and at least two gave expanded flies. These were inbred, and in the next generation, aristaless expanded flies appeared. This fact showed that the orientation of the loci was

$$\frac{a_1 \quad + \quad S}{+ \quad e_x \quad +} \text{ or } \frac{S \quad + \quad a_1}{+ \quad e_x \quad +}.$$

Two three-point backcrosses (table 18) confirmed this order and gave an aristaless expanded recombination percent of 0.05, and an expanded star recombination percent of 1.7. The data in the second experiment (right column) gave unusually little expanded star crossing over, except in one culture (the last) that gave unusually much.

TABLE 18

P_1, aristaless star×expanded; BC., F_1 star ♀×aristaless expanded ♂. (June 1925).

ARISTALESS STAR	EXPANDED	ARISTALESS EXPANDED	STAR	ARISTALESS	EXPANDED STAR	ARISTALESS STAR	EXPANDED	ARISTALESS EXPANDED	STAR	ARISTALESS	EXPANDED STAR
85	97	..	..	1	1	51	52		1	.	1
25	31	..	.	..	1	28	25			1	1
61	62	.		.	3	87	70			1	
106	86		.	1	2	109	96			.	
13	17		.	.	.	18	10		.		
116	89	.	.	4	1	46	37				
87	57	..		2	3	50	76				
27	26		.	1	2	104	114		.	7	3
17	16		.	1							
75	71					1105	1032		1	19	18

Expanded dachsous double recessive

The previous experiments had shown that aristaless and dachsous lie on the same side of star; likewise that aristaless and expanded lie on the same side of star. From this it follows that expanded and dachsous are on the same side of star.

The data of table 12 had given 1.5 percent of recombination for expanded star and 0.7 for dachsous star. Accordingly the attempt to obtain an expanded dachsous stock was made on the assumption that the locus of expanded is further from star than is the locus of dachsous. Expanded star flies were bred to dachsous and the F_1 star females were backcrossed to expanded males. The expanded not-star flies produced as the result of crossing over were inbred (4 ♀×4 ♂). It was expected that part of the expanded flies should be of the constitution

$$\frac{e_x \qquad d_s}{e_x \qquad +},$$

coming from crossing over between the loci of two recessives in the

$$\frac{e_x \qquad + \qquad S}{+ \qquad d_s \qquad +}$$

mother. The remainder should be of the constitution

$$\begin{array}{cc} e_x & + \\ e_x & + \end{array}$$

coming from crossing over between the loci of dachsous and star. If two of the first type be bred together, then the double recessive would be produced in the next generation. The inbreeding did not produce the double type. Evidently if there were present flies of the first type they had mated only with flies of the second type. The flies were then remated to dachsous, and in the offspring of some, dachsous flies appeared

$$\frac{e_x \quad d_s}{+ \quad d_s}$$

These were inbred and the double recessive was obtained. This fact showed that the order is, as assumed, expanded, dachsous, star (or star, dachsous, expanded).

It had been anticipated that there would be considerable difficulty in distinguishing the double recessive expanded dachsous from expanded, since the main characteristic of dachsous is crossveins close together, and this feature is likewise present in expanded. However, it was found that the shortness of wings in dachsous was very apparent in the double form, which had wings practically as broad as long. The expanded dachsous flies were small and rather low in viability and inclined to sterility, so that much trouble was experienced in carrying on stocks and securing crosses. This difficulty was solved by crossing expanded dachsous to curly, a second-chromosome dominant character that is lethal when homozygous and whose gene occurs in a chromosome that also carries crossover suppressors. The F_1 curly flies, when bred together, gave a stock that was mostly curly flies heterozygous for expanded dachsous with a few expanded dachsous homozygotes.

Expanded star by dachsous backcross

For determining the recombination percentages for expanded dachsous and star, an

$$\frac{e_x \quad + \quad S}{+ \quad d_s \quad +}$$

backcross was carried out (table 19). The males used in backcrossing were usually curly over expanded dachsous, and the curly offspring were discarded before making the separations for the three other characters. The data gave 0.2 as the expanded dachsous, and 1.2 as the dachsous star recombination percent.

TABLE 19

P_1, expanded star×dachsous; BC., F_1 star ♀ ×expanded dachsous curly. (*July 1925*).

EXPANDED STAR	DACHSOUS	EXPANDED DACHSOUS	STAR	EXPANDED	DACHSOUS STAR	EXPANDED STAR	DACHSOUS	EXPANDED DACHSOUS	STAR	EXPANDED	DACHSOUS STAR
121	129	1	..	3	3	17	28	..	..	1	..
57	61	..	..	..	1	48	51	..	.	1	..
58	80	..	..	2	.	53	64	..	..	..	..
42	64	..	..	.	..	49	54	1	..	..	1
52	61	..	1	.	..	56	45	..	..	.	2
44	57	.	..	1	1	61	64	.	..	4	1
60	71	..	1	.	2	62	68	.	.	1	..
30	44		..		.	51	64	.	..	1	.
65	89	.	1	1	.	55	70	..	..	.	..
46	57	..	1		..	43	66	..	..	..	2
48	67	.		1	.						
						1118	1354	2	4	16	13

THE ORDER OF THE LOCI

The three-point experiments reported in Part II allowed the order of the loci telegraph, aristaless, expanded, dachsous and star to be deduced. Dachsous was found to lie on the same side of star as telegraph

$$\frac{t_g \qquad + \qquad S}{+ \qquad d_s \qquad +},$$

table 16, as aristaless

$$\frac{a_l \qquad + \qquad S}{+ \qquad d_s \qquad +}$$

table 17, and as expanded

$$\frac{e_x \qquad + \qquad S}{+ \qquad d_s \qquad +}$$

table 19. Furthermore, in each case it was found to lie nearer to star than does each of these other loci. Similarly, expanded lies on the same side of star as does telegraph

$$\frac{t_g \qquad + \qquad S}{+ \qquad e_x \qquad +}$$

table 14 and aristaless

$$\begin{array}{ccc} a_l & + & S \\ + & e_x & + \end{array}$$

table 18 and is nearer than is either telegraph or aristaless.

Aristaless dumpy by star backcross

The final point to be determined was whether the group of the loci aristaless, telegraph, expanded and dachsous lies to the left or to the right of star. Star had been regarded as the zero point, and the other genes of the second chromosome arranged in order to the "right" of it. By using in a three-point backcross with star any member of the group in question and any locus known to be to the right of star, the relative order could be determined. Accordingly, an aristaless dumpy by star backcross was carried out (table 20). None of the more than 2000 flies of this backcross showed all three characters or were wild type. Therefore these classes constitute the double crossover classes, and the order of loci is

TABLE 20

P_1, star×aristaless dumpy; BC., F_1, star ♀×aristaless dumpy ♂. (Nov. 1925).

ARISTALESS DUMPY	STAR	ARISTALESS STAR	DUMPY	ARISTALESS	STAR DUMPY
83	105	2	2	17	16
96	93	..		19	7
111	131	..	1	14	12
113	101		.	22	16
101	137	2	2	16	16
147	164	1	..	20	9
120	107	1		12	11
147	118	1	.	12	13
918	956	7	5	132	100

$$\frac{a_1 \quad + \quad d_p}{+ \quad S \quad +}.$$

Thus, star is no longer the zero point of the second chromosome. On the basis of the data of this paper a new map may now be constructed.

CONSTRUCTION OF MAP

In table 21, the recombination data of the preceding sections have been summarized.

In determining the distances between loci two lots of data have been disregarded, namely, all recombination percentages in which the untrustworthy character telegraph is involved, (telegraph dachsous and telegraph star), and the expanded star data of table 7, that had been collected before sufficient familiarity with expanded and its interactions with star had been acquired.

TABLE 21

*Summary of recombination data**.

LOCI	EXPERIMENT	TABLE NO.	TOTAL	RECOMBINATIONS	PERCENT RECOMBINATION
Telegraph dachsous	[tel. star/dach.	16	1453	2	0.1]
Telegraph star	[tel./star	1	274	3	1.1]
	[tel /star	2	941	5	0.5]
	[tel./star	8	1169	11	1.0]
	[tel. star/exp.	14	1835	25	1.4]
	[tel. star/dach.	15	552	7	1.3]
	[(tel. star/dach.	16	1453	18	1.2)]
Aristaless expanded	aris. star/exp.	18	2175	1	0.05
Aristaless dachsous	aris.star/dach.	17	3912	12	0.3
Aristaless star	aris./star	3	334	3	0.9
	aris./star	9	4337	58	1.3
	aris.dumpy/star	20	2118	12	0.6
	Total		6789	73	1.1
	(aris.star/exp.	18	2175	37	1.7)
	(aris.star/dach.	17	3912	46	1.2)
	(Total		6087	83	1.4)
	Grand total		12876	156	1.2
Expanded dachsous	exp. star/dach.	19	2507	6	0.2
Expanded star	[exp /star	7	1346	54	4.0]
	exp./star	11	3060	46	1.5
	tel /exp star	13	2164	21	1.0
	Total		5224	67	1.3
	(exp star/dach.	19	2507	35	1.4)
	Grand total		7731	102	1.3
Dachsous star	dach./star	10	4269	31	0.7
	tel star /dach.	16	1453	16	1.1
	aris. star/dach.	17	3912	34	0.9
	exp. star/dach.	19	2507	29	1.2
	dach.^{3}/star	6	396	3	0.8
	Total		12537	113	0.9
Star dumpy	aris dumpy/star	20	2118	232	11.0

*The data enclosed by square brackets [] are considered untrustworthy. The data enclosed by parentheses () are "secondary" and are not used in calculating distances.

There is no certain evidence as to the relative order of aristaless and telegraph. But since aristaless is a trustworthy character that will be used extensively, and telegraph is relatively very poor, aristaless has been chosen as the zero of the new map. The locus of telegraph is probably of the order of 0.1 unit distant, and has been arbitrarily put to the right at 0.0±.

The aristaless expanded recombination percent was 0.05, based on 2715 flies. Since at least two flies in the 29 tested in securing the aristaless expanded double recessive, proved to be crossovers between aristaless expanded, an independent calculation of the aristaless expanded distance can be made ($2 : 29 :: x : 1.3; x = 0.09$). In view of the high probable errors of such small percentages, an accuracy to 0.1 unit is beyond reach, so the nearest tenth, namely, 0.1, has been taken as the locus of expanded.

The next locus to the right is dachsous, which is 0.3 to the right of aristaless (3912 flies) and 0.2 to the right of expanded (2507 flies). These two determinations agree at the value +0.3 as the locus of dachsous.

The dachsous star data (12,537 flies) place star at 0.9 to the right of dachsous, or at 1.2 (0.3+0.9). The expanded star data (5,224) place it at 1.3 to the right of expanded, or at 1.4 (0.1+1.3). The aristaless star data (6,789) place star at 1.2 (0.0+1.2). The weighted average of these data places the locus of star at +1.3 (to the nearest tenth).

The final map is given in figure 1.

SUMMARY AND EVALUATION OF THE MUTANTS

The serial order and relative distances have been established for a group of five mutant loci, namely, star, telegraph, aristaless (aristaless^2), dachsous (dachsous^2, dachsous^3) and expanded. These linkage relations are expressed in the map given in figure 1, in which aristaless is the new zero point of the second chromosome and the locus of star is at 1.3.

The most useful of these mutants is star, primarily because of its dominance, which enables the mutant to be followed through extended and complex experiments with a minimum of labor in preparing stocks.

The next most useful mutant is aristaless, primarily because its locus is the one furthest to the left in the map. A second advantage is that it can be used more successfully than star with plexus, which is a very important mutant and which is partly suppressed by star. A disadvantage of aristaless is that its viability is poorer than that of star, being roughly 90 percent that of the wild type. In speed of classification aristaless is very high, in which respect star is fairly poor.

Dachsous had the best viability (99 percent) of any of the mutants at the left end, and dachsous^2 is probably slightly better even than dachsous. The classification of each is certain and fairly rapid. The disadvantage of the dachsous mutants is that dumpy, which is the most important mutant between star and black, also has crossveins that are close together, and it is probable, though not definitely known, that dachsous would be unworkable with dumpy. Dachsous^3 has very poor viability (50 percent) and hence will not be used except in situations in which its female-sterility is an advantage.

Expanded offers no advantages over aristaless or dachsous, though its viability is high (97 percent) and its general characteristics good.

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SOME NUMERICAL RESULTS OF SELECTION UPON POLYHYBRIDS[1]

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INTRODUCTION

It is a well known fact that those portions of the human population that have distinguished themselves in intellectual pursuits reproduce much less freely than do the remainder of the population. The question arises: What effect has this on the population as a whole? It is generally assumed that this adverse selection lowers the general level of intellectual

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power, so that a steady deterioration in that respect is in progress. Is this correct, and if so, at what rate does the deterioration occur? Or is it possible, as some have suggested, that intellectual power is due to the unusual combinations of genes which continually arise in the general population, and that the frequency of these combinations is little affected by the fact that the individuals resulting from them do not propagate freely? How much is it affected?

How can this proble m be attacked? In man the common individual differences are generally multiple factor cases whose elements are unknown to us (MORGAN 1924). Intellectual power is doubtless due to special combinations of numerous factors; the problem is: If those special combinations do not propagate freely, what effect has this on the frequency of occurrence of such combinations? To obtain the general principles here at work, the problem must first be made definite and simplified; basing on the results thus obtained, the more complex problems may be attacked. We may first examine the effect produced when a particular combination of several factors is completely excluded from further propagation. For example, what is the effect on the occurrence of a characteristic that is due to the simultaneous presence of m dominant factors, if the individuals which have all these factors do not breed further? Or what is the effect if a given characteristic is due to the simultaneous occurrence of m particular dominant factors and n particular recessive ones, and the individuals with this combination do not further breed? Or suppose that some definite proportion--say one-half—of them breed while the rest do not?

As perhaps the simplest case, let us take that in which the characteristic in question depends upon the occurrence together of a certain number of dominant factors. An instance of this sort is found in the agouti color of rats, guinea pigs, and rabbits (CASTLE and WRIGHT 1916). What is the effect upon a population, heterozygous as to m independent factors which determine the agouti color, when, in the F_2 and all subsequent generations, no agouti individuals are allowed to breed, and the rest of the population breed at random? An answer to the above question, even if generalized to fit any number of factors, would by no means solve our original problem. In the human stock the problem may be complicated by such factors as (1) linkage, (2) selective mating, (3) the fact that the superior individuals (in question) do not fail to reproduce entirely but only reproduce at a slower rate, and (4) the possibility that some of the factors involved are recessive, others dominant. However, the solution of the problem of the effect upon a polyhybrid stock of eliminating in every generation all individuals who are dominant for all factors concerned will perhaps form a basis upon which the more complex problems may be attacked.

The greater part of the work done to date upon the effects of various schemes of selection has been upon monohybrid stocks. PEARSON (1904) showed that the several types in a Mendelian population in which the F_2 generation was composed of 25 percent *aa*, maintains a constant proportion under conditions of random mating and no selection. JENNINGS (1916) derived a number of formulæ showing the results of various systems of breeding in monohybrids. WARREN (1917) showed that the elimination of a homozygous type in a monohybrid under conditions approximating those of natural selection, caused a progressive decrease in the number of such individuals appearing. HALDANE (1924) found mathematical expressions for the effect of selection on simple Mendelian populations mated at random. JENNINGS (1917) showed the effect of diverse systems of breeding, with respect to two pairs of characters. WRIGHT (1921) demonstrated that, in a polyhybrid stock, when dominance was lacking, the two extreme types were the only ones which could be permanently fixed.

In the present paper formulæ are derived for obtaining the results of the following systems of selection.

I. *The elimination in each generation of all individuals dominant for all of the m independent factors dealt with in a polyhybrid of m allelomorphs.* The value of *m* in this case must be less than 5. While it is relatively an easy matter to derive formulæ for higher hybrids the formulæ soon become too cumbersome, since they are for deriving any generation in terms of the preceding generation.

II. *The elimination in each generation of all individuals who are not dominant for all of the m independent factors in a polyhybrid of m allelomorphs.* These formulæ are in terms of *n*, the number of the filial generation: that is, in order to obtain the proportion of the types appearing in any generation it is not necessary to know the percentage in any other generation.

III. *The elimination in each generation of all individuals recessive for all m independent factors in a polyhybrid of m allelomorphs.* These formulæ are also in terms of *n*, where *n* is equal to *2* and *3*; when *n* is larger each generation will have to be derived in terms of the preceding generation.

IV. *The elimination in each generation of all individuals* (*A*) *containing two or more independent dominant factors, and* (*B*) *containing three or more independent dominant factors in a polyhybrid of m allelomorphs.* The formulæ are for deriving any generation in terms of the preceding generation.

Tables showing the percentages of the given types appearing in each generation are given.

PART I.

THE ELIMINATION IN EACH GENERATION OF ALL INDIVIDUALS DOMINANT FOR ALL OF THE m INDEPENDENT FACTORS DEALT WITH IN A POLYHYBRID OF m ALLELOMORPHS

How rapidly will the completely dominant individuals disappear from the population if none of such dominants produced leave progeny?

In dealing with this problem, we shall first deal with cases involving but few pairs of factors, then with more complex cases. By comparison of the results for successively larger number of factors, we shall develop a method applicable to polyhybrids with any number (m) of allelomorphs. In transforming one generation into the next, we shall use throughout the general method introduced by JENNINGS (1917), of dealing primarily with the gametes, the zygotes of any given generation being readily obtainable from the knowledge of the gametes that produce them. This method reduces enormously the complexity of the problems, as compared with working primarily with the zygotes. In the present paper, however, this method is further greatly simplified in such a way as to make it a practicable one for dealing with polyhybrids of many pairs of allelomorphs.

Designation of the zygotic and gametic generations

We begin in each case with a completely heterozygotic parent, as *Aa Bb*, produced by crossing *ABAB* with *abab*. This original heterozygote we shall call the F_1 generation. The gametes produced by the F_1 generation of zygotes we shall call the G_1 gametes; these produce by their mating the F_2 zygotes, these the G_2 gametes, and so on. The order of alternate zygotic and gametic generations is thus:

$F_1 \rightarrow G_1 \rightarrow F_2 \rightarrow G_2 \rightarrow F_3 \rightarrow G_3$, and so on.

1. *The dihybrid*

In the progeny of the monohybrid, *Aa*, of course one elimination of the dominant zygotes gets rid completely of the dominant factor *A*, leaving only recessives. For the dihybrid *AaBb*, the problem of determining the constitution of each generation in terms of that preceding it, in case all the individuals (zygotes) dominant for both *A* and *B* are eliminated in each generation, may be attacked by the method of JENNINGS (1917).

The original parents are *AABB* and *aabb*, yielding by their cross the F_1 generation *AaBb*. These F_1 individuals produce in equal numbers the four sorts of G_1 gametes: *AB*, *Ab*, *aB*, and *ab*. Call the respective proportions of these *P*, *Q*, *R*, and *S*, then the proportions of the different kinds of zygotes yielded by the random mating of these four sorts of gametes (as shown in the usual dihybrid table of zygotes) are given by the various terms of the expansion of $(P+Q+R+S)^2$. They are the following:

TABLE 1

Dihybrid.

ZYGOTES OF F_2		IN TERMS OF GAMETE G_1 (PRODUCED BY F_1)
a $(=AABB)$	=	P^2
b $(=2AABb)$	=	$2PQ$
c $(=AAbb)$	=	Q^2
d $(=2AaBB)$	=	$2PR$
e $(=4AaBb)$	=	$2PS+2QR$
f $(=2Aabb)$	=	$2QS$
g $(=aaBB)$	=	R^2
h $(=2aaBb)$	=	$2RS$
i $(=aabb)$	=	S^2

Designate the proportions of each of these nine kinds of zygotes respectively by the letters *a* to *i*, as shown in table 1; these proportions, in terms of the proportions of the gametes from which they are formed, are given at the right. Now from these zygotes we eliminate all double dominants—those containing *AB*; that is, we eliminate the zygotes designated *a*, *b*, *d*, and *e*, leaving only *c*, *f*, *g*, *h*, and *i*. If each of these produces two gametes, the natural proportions will be retained; we shall then find that the proportions *P*, *Q*, *R*, and *S* of the four sorts of gametes produced are those given in the second column of table 2.

TABLE 2

(1) GAMETES OF G_2 (FROM F_2)	(2) IN TERMS OF PROPORTIONS OF ZYGOTES OF F_2	(3) IN TERMS OF PROPORTIONS OF GAMETES OF G_1 (FROM F_1)
P $(=AB)$	0	0
Q $(=Ab)$	$2c+f$	$2Q^2+2QS$
R $(=aB)$	$2g+h$	$2R^2+2RS$
S $(=ab)$	$f+h+2i$	$2QS+2RS+2S^2$

We have now but to transform the proportions of the zygotes of F_2, given in the second column into those of the gametes of G_1 (as shown in table 1), in order to have the proportions of the gametes of G_2 stated in terms of the proportions of the gametes of G_1; these are given in the third column of table 2. Since it is only the relative proportions that we require, the factor 2 may be taken out, yielding table 3 as our basic table by which, from the proportions of the gametes of one generation, the proportions of those of the next generation may be derived.

TABLE 3

Dihybrid. Proportions of the gametes of generation $n+1$, in terms of those of generation n, in case all zygotes having both factors dominant have been eliminated.

GAMETES OF GENERATION $n+1$	IN TERMS OF THOSE OF GENERATION n
P_{n+1}	0
Q_{n+1}	$Q_n\ (Q_n+S_n)$
R_{n+1}	$R_n\ (R_n+S_n)$
S_{n+1}	$S_n\ (Q_n+R_n+S_n)$

When we start with the F_1 dihybrid *AaBb*, the four sorts of gametes of G_1, produced by it, are numerically equal; application of table 3 therefore yields the following proportions for successive generations:

TABLE 4

Dihybrid.

GAMETES OF				
G_1	G_2	G_3	G_4	G_5
$P = 1$	0	0	0	0
$Q = 1$	2	10	310	368,510
$R = 1$	2	10	310	368,510
$S = 1$	3	21	861	1,281,641

At any generation desired, the proportion of the different sorts of zygotes is obtained by the use of table 1. What we desire to know is the proportion in each generation of the zygotes that contain the double dominant *AB*, and are hence to be eliminated. In the F_2 generation, all zygotes into which the gamete $P(=AB)$ enter are eliminated, and no such gametes or zygotes again appear. In these later generations, the total proportions of the different sorts of zygotes are therefore given by the several terms of the expansion of $(Q+R+S)^2$. The double dominants are now formed only as the combination $c(=AaBb)$ of table 1, and in the proportion $2QR$ (since the combination $2PS$ of table 1 no longer appears). The proportions of the double dominants and of the other combinations for any zygotic generation are therefore obtained from the proportions of the gametes yielded by the previous generation, by table 5.

TABLE 5

Dihybrid.

ZYGOTES OF GENERATION $n+1$	IN TERMS OF GAMETES FROM GENERATION n	EXAMPLE ZYGOTES IN GENERATION F_4	
		TOTAL	PERCENT
Containing *AB*	$2QR$	200	11.9
Not containing *AB*	$Q^2+R^2+S^2+2QS+2RS$	1481	88.1
Total	$(Q+R+S)^2$	1681	100

As an example, the zygotic proportions in F_4 (as derived from table 4) are given at the right.

Simplification of the method, by the use of common designations for all gametes containing the same number of dominant factors: The above method can be greatly simplified (as seen particularly in work with numerous allelomorphs) by the following consideration.

1. All different kinds of gametes having the same number of dominant (and therefore of recessive) factors such as *Ab* and *aB*, are similarly affected by any form of selection that eliminates all zygotes showing a certain specified number of dominant factors, *irrespective of which particular factors there are.*

2. Therefore, if at the beginning these diverse kinds of gametes are present in equal proportions, their proportions remain equal throughout the entire selective breeding. This is actually the case when we begin, as in the present work, with a parent that is heterozygotic for all its factors.

3. Such equal groups of gametes can be given the same designation, and the proportion of each can then be determined by the one operation of determining the proportion corresponding to this designation. Thus the number of diverse unknown quantities with which we must work is greatly reduced. The total number of different kinds of gametes produced by a zygote that is heterozygotic for m factors is 2^m: by the procedure just mentioned this reduced to but $m+1$ different classes. For our purposes one kind of gamete—that in which all the factors are dominant—does not appear at all; the reduction is therefore from 2^m-1 kinds to m classes. While in the dihybrid the reduction is thus only from 3 unknown quantities to 2, in the octahybrid the reduction is from 255 to 8, bringing the treatment of these and still more complex cases within the bounds of practicability.

In accordance with these principles, therefore, in the dihybrid the proportion of each of the two gametes *Ab* and *aB* (which we have called *Q* and *R*) can be given a single designation (q). In the tetrahybrid the proportions of the four gametes *ABCd*, *ABcD*, *AbCD*, and *aBCD* will likewise be given a common designation (q).

Designation of gametes: For further development of the matter, it is desirable to have some uniform system of designating the proportions of the different classes of gametes. Suppose that we begin with a parent that is a polyhybrid, *AaBbCc*, etc., for m pairs of factors. Then the diverse gametes will contain respectively m, $m-1$, $m-2$ . . . . , to 0 dominant factors. We shall employ for these respectively the designations given below: (table 6)

TABLE 6

Designation of Gametes.

No. of dominant factors...... ..	m	$m-1$	$m-2$	$m-3$	$m-4$	and so on
Designation of gamete	p	q	r	s	t	and so on

Thus in a dihybrid $AB=p$, Ab and aB each $=q$, $ab=r$; in a tetrahybrid $ABcd=r$; in a hexahybrid, $aBCdeF=s$, and so on.

Since in the dihybrid, as just set forth, the proportions of each of the two gametes that are designated Q and R in table 2 are now to be designated q, while the one there designated S is to be called r, table 3 assumes the abridged form given in table 7.

TABLE 7

Dihybrid.

GAMETES OF GENERATION $n+1$	IN TERMS OF GAMETES OF GENERATION n
p	0
q	$q(q+r)$
r	$r(2q+r)$

(where q = proportion of Ab or of aB; r = proportion of ab)

By making the same substitution in table 5, we find that the proportion of double dominant zygotes (AB) present (and to be eliminated) in any generation is (in terms of the gametes which produce that generation):

$$\frac{2q^2}{(2q+r)^2} \tag{8}$$

Formula 8 makes it possible to determine with some labor for a series of successive generations the proportion of completely dominant individuals remaining, and the curve of their rate of disappearance.

A formula for obtaining at once the gametic proportions in any generation G_n (and thence the zygotic proportions), without determining it for the intervening generations, would be extremely useful. An approximate formula of this sort may be derived in the following way, for which I am indebted to Doctor F. D. MURNAGHAN of the Mathematics Department of the JOHNS HOPKINS UNIVERSITY. In table 7, at the right, let r/q for generation n be represented by l_n: then on dividing the first equation by the second, we obtain

$$l_{n+1}=\frac{ln(2+l_n)}{1+l_n}=1+l_n-\frac{1}{1+l_n} \tag{9}$$

Substituting the values of q and r, the same proportions are yielded for the successive generations as are given in table 4.

In formula 9, when n becomes large, it is found that the value of l_n approaches $n-1$. Now our ratio

$$\frac{2q^2}{(2q+r)^2}$$

of formula 8, if expressed in terms of l_n, becomes

$$\frac{2}{(2+l_n)^2}$$

Substituting, in this expression, for l_n the value $n-1$ we obtain for the dihybrid as the proportion of double dominants in any generation n (when n is large) the following:

$$\frac{2}{(1+n)^2} \tag{10}$$

Where n is small this equation of course cannot be used.[1] Experience shows that if we place in the denominator the quantity $-\sqrt{n/2}$, yielding the formula

$$\frac{2}{\left(n-\sqrt{\frac{n}{2}}+1\right)^2} \tag{11}$$

the results come throughout, when $n>7$, extremely close to the correct values obtained by the repeated use of table 7, and formula 8. (See table 35.)

2. *The trihybrid*

The problem of the results of eliminating in each generation all the completely dominant individuals (those containing ABC) in the trihybrid, is attacked by the same methods employed for the dihybrid. We begin with the F_1 triple heterozygote $AaBbCc$. This produces in equal numbers 8 sorts of gametes of G_1 that are represented in table 12. We may designate these eight kinds provisionally by the first eight letters of the alphabet,

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[1] The problem of deriving the gametic proportion in any generation (G_n) from the formulæ

$$q_{n+1}=q_n(q_n+r_n)$$
$$r_{n+1}=r_n(2q_n+r_n)$$

without determining it for all previous generations was proposed by MURNAGHAN (1923) in THE AMERICAN MATHEMATICAL MONTHLY. An approximate solution in terms of n has been given by BENNETT (1924).

a to h (these designations are later to be discarded). These eight fall into 4 groups, containing respectively 3,2,1, and 0 dominant factors, as shown in the table. In accordance with the principles set forth on an earlier page, the proportions of any one kind of gamete of each of these groups may be given a common designation: in accordance with table 6, the groups will be p, q, r, and s, as shown in the lower row of table 12.

TABLE 12

Gametes (G_1) from the trihybrid AaBbCc with their designations and classifications into groups.

1. No. of dominant factors	3	2	1	0
2. Designations and consitution of different kinds . . .	*a. ABC*	*b. ABc* *c. AbC* *d. aBC*	*e. Abc* *f. aBc* *g. abC*	*h. abc*
3. Number of kinds of gametes in each group	1	3	3	1
4. Designation of the gametes according to groups	p	q	r	s

The random mating of these 8 gametes produces in the F_2 generation 64 zygotes, of which 27 contain ABC and are therefore eliminated.

To obtain the proportions of complete dominants in the later generations, we may proceed on the following considerations. In F_2, all the zygotes into which the gamete $p(=ABC)$ enters are eliminated, and in later generations that gamete does not again enter into the formation of zygotes. We may therefore omit this gamete from consideration, determining only the proportion of complete dominants (to be eliminated) that result from the mating of the other three groups. In producing the F_2 generation, these three groups taken by themselves produce precisely the zygotes which are not eliminated by the process of removing all those into which enters the gamete $p(=ABC)$. We may represent these three groups thus: $3q+3r+s$. The total number of zygotes of F_n which their mating produces s then $(3q+3r+s)^2$.

If we designate each of these gametes separately by the provisional designations given at 2, of table 12, they yield in mating zygotes whose proportions are given by the expansion of $(b+c+d+e+f+g+h)^2$. Detailed study will show that the following contain the three dominants ABC

$$2bc,\ \ 2bd,\ \ 2bg,\ \ 2cd\ \ 2cf,\ \ 2de$$

Replacing these letters by their group equivalents (lower row of table 12), we obtain for the zygotes containing dominants (and hence to be eliminated):

$$6q^2+6qr;\ \text{or}\ 6q(q+r) \qquad (13)$$

In the gametes from the F_1 heterozygote, $AaBbCc$, the value of each of the designations q, r, and s is 1. In later generations these values will be different, but the expression (13) remains correct when the proper values are substituted for q, r, and s. Thus in any generation the proportion of zygotes eliminated, in terms of the gametes from whose mating they are derived, is:

$$\frac{6q(q+r)}{(3q+3r+s)^2} \tag{14}$$

In order to simplify the problem of expressing the gametes of any generation in terms of those of the preceding generation it is necessary that we ignore for the time being certain very important factors, which will later be evaluated. The gametes will first be treated as if they were immutable units, that is, that the gametes which fuse to form a zygote appear unchanged in the next generation—that a zygote can produce only the gametes out of which it itself was formed —as if linkage between all factors were complete. This assumption is of course true when the zygote is either homozygous or heterozygous for a single pair of factors. When, however, the zygote is heterozygous for two or more factors certain rearrangements of genes occur so that some of the gametes produced in the next generation are different from those which formed the zygote in question. For example, in the trihybrid the gametes *aBc* and *abC* are represented by r. The zygote *aaBbCc* formed from them would be represented by r^2. This zygote could produce gametes q(*aBC*) and s(*abc*) as well as r(*aBc*) and (*abC*). This "shifting" of factors will be treated later.

Now, by methods parallel to those employed for obtaining tables 3 and 7 in the dihybrid, we obtain, for the trihybrid, table 15 for transforming the gametic proportions of one generation into those of the next.

TABLE 15

Trihybrid (Treating the gametes as units). Proportions of the different classes of gametes of generation $n+1$, in terms of those of the gametes of generation n.

GAMETES OF $n+1$	IN	TERMS OF GAMETES OF n
q	=	$q(q+2r+s)$
r	=	$r(2q+3r+s)$
s	=	$s(3q+3r+s)$

3. *The tetrahybrid*

By application of the methods set forth for the di- and trihybrid, the problem of the results of eliminating all completely dominant zygotes in tetrahybrids may be solved. Only the essential data and results will be given. The number of groups of gametes with which we must now work is 4; let the proportions of these be designated q, r, s, and t according as they contain respectively 3, 2, 1, and 0 dominant factors. The proportions of these derived from generation $n+1$, expressed in terms of those derived from the previous generation n are given in table 16.

TABLE 16

Tetrahybrid (treating the gametes as units). Proportions of any class of gametes belonging to groups q, r, s, and t from generation n+1, in terms of those from generation n.

GAMETES OF GENERATION $n+1$		IN TERMS OF GAMETES OF GENERATION n
q	=	$q(q+3r+3s+t)$
r	=	$r(2q+5r+4s+t)$
s	=	$s(3q+6r+4s+t)$
t	=	$t(4q+6r+4s+t)$

In the tetrahybrid the proportion of completely dominant zygotes (hence to be eliminated), in any generation is found to be in terms of the proportions of the gametes which produce them:

$$\frac{4q(3q+6r+2s)+6r^2}{(4q+6r+4s+t)^2} \tag{17}$$

4. *Polyhybrids for any number m pairs of allelomorphs*

Comparative consideration of results thus far reached with the di-, tri-, and tetrahybrids now puts us in a position to obtain by mathematical induction a general method for dealing with any polyhybrid, whatever the number of pairs of allelomorphs, thus yielding a general solution of our problem.

a. General formulæ for the proportions of the different types of gametes of any generation $n+1$, in terms of those of the previous generation n;

In any polyhybrid *AaBbCc* . . . . , heterozygous for all its m pairs of allelomorphs, the number of different kinds of gametes produced is 2^m. If these be grouped according to the number of dominant factors they contain, in order from m dominants to 0, we find that they fall into $m+1$ groups, and the numbers of different gametes in each group are equal to the successive terms of the expanded binomial $(1+1)^m$. This has been illustrated for the trihybrid in table 12. When now these gametes mate among themselves at random, each kind mates with all the groups (table 12); the consequence is that in the next generation all fall again into the form of the expanded binomial $(1+1)^m$. In our problem, however, as we have seen, the first group (that containing m dominants) does not occur after the first selection has occurred. This modifies the binomial series in definable ways, which may be discovered by comparing the gametic formulæ for the di-, tri-, and tetrahybrids given in tables 7, 15, and 16.

These formulæ give the proportions of the different types of gametes of any generation $n+1$, in terms of those of the previous generation n.

It is most instructive to compare first the formula for any gametic type (r) containing just $m-2$ dominants. These are (considering the gametes as fixed units):

TABLE 18

Linkage Complete.

m	GENERATION $n+1$	IN TERMS OF GENERATION n
2	r	$r(2q+r)$
3	r	$r(2q+3r+s)$
4	r	$r(2q+5r+4s+t)$

The expressions within the parentheses, at the right, consist of a set of terms that contain the series of gametes q, r, s, etc., (these being the designations for the gametes containing respectively $m-1$, $m-2$, $m-3$ . . . . 0, dominants), each with a coefficient. These coefficients show a relation to the expanded binomial $(1+1)^m$ that may be defined as follows. Each is the expansion of $(1+1)^m$, from which has been subtracted, beginning at the left, the terms of $(1+1)^{m-2}$. Thus to illustrate from the trihybrid $(m+3)$:

$$\begin{array}{ll} (1+1)^m & =1p+3q+3r+1s \\ (1+1)^1 & =1p+1q \\ \hline \text{Difference} & \quad 2q+3r+1s \end{array} \tag{19}$$

Now, further comparison will show that this relation is general, the expansion to be subtracted being always $(1+1)^{m-k}$, where $m-k$ is the number of dominant factors in the gamete whose proportions are sought. Thus, for the gamete r, containing $m-2$ dominant factors, in the case of the tetrahybrid, we have

$$\begin{array}{ll} (1+1)^4 & =1p+4q+6r+4s+1t \\ (1+1)^2 & =1p+2q+1r \\ \hline \text{Difference} & \quad 2q+5r+4s+1t \end{array} \tag{20}$$

Thus the coefficients are always given by the successive terms of $(1+1)^m-(1+1)^{m-k}$, the subtraction being at the left. We may therefore formulate as follows:

FORMULA 21

In any polyhybrid of m pairs of allelomorphs:

Let the gametes containing respectively m, $m-1$, $m-2$, $m-3$, ... 0 dominant factors be designated by the series of letters p, q, r, s, t, ... z (so that r proportion of any type of gametes containing $m-2$ dominants, etc.).

Let x represent algebraically any one of these types containing $m-k$ dominant facors.

Let c_1, c_2, c_3, c_4, etc., represent the respective coefficients of p, q, r, s, etc.

Then if all completely dominant zygotes are eliminated in each generation, the proportions of the gametes, if linkage were complete, of any type x, containing $m-k$ dominant factors, for any succeeding generation $n+1$, would be given in terms of the gametic proportions of the preceding generation n, by an expression of the form.

$$\begin{array}{ccc} \text{Generation } n+1 & & \text{Generation } n \\ x & = & x(c_2q+c_3r+c_4s \;.\;.\;.\;.\; \text{etc.}) \end{array} \tag{21}$$

in which the successive values of the coefficients c_2, c_3, c_4, etc., are given by the terms of the series $(1+1)^m-(1+1)^{m-k}$ (the subtraction beginning at the left). The coefficient c_1 of course always disappears.

By the use of this general formula 21, the special formulæ for transforming the gametes of any generation n into those of the succeeding generation $n+1$ can be at once written out for any polyhybrid.

Example. In a tetrahybrid, what is the formula for a gametic type r containing just two dominants, (linkage complete) in terms of the gametes of the preceding generation? Here $m=4$, $k=2$, $m-k=2$. The coefficients are therefore given by $(1+1)^4-(1+1)^2$, and the formula is:

$$\begin{array}{ccc} \text{Generation } n+1 & & \text{Generation } n \\ r & = & r\,(2q+5r+4s+t) \end{array}$$

Similarly for the octahybrid, what is the formula for the gametic type u, containing just three dominants? Here $m=8$, $m-k=3$. The coefficients are given by $(1+1)^8-(1+1)^3$, so that the formula is:

$$\begin{array}{ccc} \text{Generation } n+1 & & \text{Generation } n \\ u & = & u(5q+25r+55s+70t+56u+28v+8w+x) \end{array}$$

We have now derived a general method for obtaining the gametes of generation G_n+1 in terms of the gametes of generation G_n which holds as long as none of the combinations of gametes are heterozygous in more than a single pair of characters. We will now determine what modifications are necessary to make the method hold for all gametes regardless of the number of factors in which the combinations are heterozygous. To do this we must first consider the effects of this "shifting" or recombination of factors within the zygote, which results in the zygote producing gametes quite different from those from which it itself was formed.

It will be noted that as a result of the reduction division gametes are always produced in pairs. Either gamete may have all or none of the dominant factors (hence also recessive) of the zygote or any number of the same; however, *the pair of gametes will together have exactly as many dominant and recessive factors as the parent zygote had.* It follows that no matter what gametes are produced from a zygote they will be produced in pairs and the two gametes of the pair will contain together just as many dominant and recessive factors as the gametes of any other pair. To illustrate: a pentahybrid zygote has four different dominant factors which are all heterozygous. Let it be represented thus *AaBbCcDdee.* There will be 16 different gametes produced which will all occur in pairs designated by our scheme as *qu*, *rt*, *ss*. As these pairs are the only ones which contain these particular factors, of necessity, the parent zygote was formed by the fusion of the members of one of these pairs. Thus the gametes of any one of these pairs in the G_n generation produce all three pairs in generation G_{n+1}. Or stated conversely

$$qu_{n+1} = qu_n + rt_n + ss_n$$

and so on for each of the other pairs.

Pairs of gametes then function in groups. A pair whose sum of dominant factors in generation G_{n+1} was, say $m-1$, is produced by all pairs which had $m-1$ dominants in generation G_n and by no other pair.

Our method of designating the gametes by a symbol which stands for all gametes which contain a like number of dominant factors allows us to make a general statement covering all of this "shifting" of factors, so we can state exactly what gametes will be produced in generation G_{n+1} from given gametes of generation G_n.

Let us take for example the zygotes of a generation of a pentahybrid. We will form the zygotes by crossing every gamete with itself and every other gamete, thus:

TABLE 22

p	q	r	s	s	et	pu
p	q	pq	pr	s	pt	qu
q	qp	qq	qr	qs*	qt*	qu*
r	rp	rq	rr*	rs*	rt*	ru*
s	sp	sq*	sr*	ss*	st*	su*
t	tp	tq*	tr*	ts*	tt*	tu
u	up	uq*	ur*	us*	ut	uu

Ignoring all zygotes containing *p* as a factor, for according to our scheme of selection no gametes containing all *m* dominants (*p*) are produced, if we mark all zygotes with an asterisk as shown above, each line of asterisks will cross all pairs of gametes which contain an equal number of dominant factors, and hence each pair will produce in the next generation all of the gametes on the line which crosses it, paired as they are in the diagram, and produce no other gametes. Starting with the pair which contains the greatest number of dominants the groups are:

NO. OF DOMINANT FACTORS IN ZYGOTE	PAIR OF GAMETES PRODUCED	
2 (m−1)	*qq*	
2 (m−1)−1	*qr, rq*	
2 (m−1)−2	*qs, rr, sq*	
2 (m−1)−3	*qt, rs, sr, tq*	
2 (m−1)−4	*qu, rt, ss, tr, uq*	(23)
2 (m−1)−5	*ru, st, ts, ur*	
2 (m−1)−6	*su, tt, us.*	
2 (m−1)−7	*tu, ut*	
2 (m−1)−8	*uu.*	

The above can be simplified by combining the like pairs. Thus as $qr = rq$ they can both be written either as one or the other.

We have now determined which gametes are produced in the G_{n+1} generation from a given pair of gametes in generation G_n. The next point to be settled is in what proportion are the various gametes produced?

First, when a pair of like G_n gametes produce a homozygous zygote, all of the gametes produced in G_{n+1} are, of course, identical and the same as the gametes G_n. This is the case with all zygotes which have $2\ (m-1)$ dominant factors paired as they are in the diagram. Second, when a pair of G_n gametes produce a zygote heterozygous for only one factor there are only 2 kinds of gametes produced in the G_{n+1} generation and these are produced in equal numbers and are identical with the two G_n gametes. This is the case with all zygotes which have $2\ (m-1)-1$ dominant factors. Third, when a pair of G_n gametes produce a zygote heterozygous for two factors, the case is more complicated, for here 4 different gametes are produced in generation G_{n+1} only 2 of which were present in the direct line in generation G_n. For example the zygote *AABbCcdd* will produce four gametes paired as follows:

$$\left\{\frac{ABCc}{Abcd}\right\} \quad \left\{\frac{ABcd}{AbCd}\right\} \quad \left\{\frac{AbCd}{ABcd}\right\} \quad \left\{\frac{Abcd}{ABCd}\right\}$$

According to our scheme these would be written *qs*, *2rr*, *sq*. As the different pairs of gametes are produced in equal numbers the number of a particular gamete produced by all such zygotes is ¼ of the number of such pairs produced. For example

$$q_{n+1} = \frac{q_n s_n + 2r_n r_n + s_n q_n}{4}$$

Fourth, when a zygote produced by a pair of G_n gametes is heterozygous in regard to these factors, eight G_{n+1} gametes are produced. Thus the zygote *AaBbCcdd* produces the following pairs of gametes:

$$\left\{\frac{ABCd}{abcd}\right\}\left\{\frac{ABcd}{abCd}\right\}\left\{\frac{AbCd}{aBcd}\right\}\left\{\frac{aBCd}{Abcd}\right\}\left\{\frac{Abcd}{aBcd}\right\}\left\{\frac{aBcd}{AbCd}\right\}\left\{\frac{abCd}{ABcd}\right\}\left\{\frac{abcd}{ABcd}\right\}$$

These would be represented:

$$qt,\ 3rs,\ 3sr,\ tq$$

The relative number of a given gamete in generation G_{m+1} would be 1/8 of the total number of such zygotes. For example:

$$q_{m+1}=\frac{q_nt_n+3r_ns_n+3s_nr_n+t_nq_n}{8}$$

Fifth, when a zygote is heterozygous for four factors, sixteen different gametes are produced. They are represented by

$$qu,\ 4rt,\ 6ss,\ 4tr,\ uq.$$

Each gamete would be 1/16 of the total. Thus

$$q_{m+1}=\frac{q_nu_n+4r_nt_n+6s_ns_n+4t_nr_n+u_nq_n}{16}$$

We can now state the above cases in a general form. Let h = the number of heterozygous factors in a zygote. Then the number of any gamete g produced by any zygote would be expressed

$$g=\frac{(1+1)^h}{2^h}$$

where the expanded form of the numerator would give the coefficients of the pairs of gametes in the order of their occurrence in table 22. A further step in simplification can be taken by combining like pairs and cancelling factors that occur in both numerator and denominator. To illustrate:

TABLE 24

VALUES OF h	G_{n+1} GAMETES		ZYGOTES IN TERMS OF G_n GAMETES		SIMPLIFIED G_n GAMETES
1	q_{n+1}	=	$\frac{q_nr_n+r_nq_n}{2}$	=	q_nr_n
2	q_{n+1}	=	$\frac{q_nr_n+2s_ns_n+r_nq_n}{4}$	=	$\frac{q_nr_n+s^r_n}{2}$
3	q_{n+1}	=	$\frac{q_nt_n+3r_ns_n+3s_nr_n+txqx}{8}$	=	$\frac{q_nr_n+3r_ns_n}{4}$
4	q_{n+1}	=	$\frac{q_nu_n+4r_nt_n+6s_ns_n+4t_nr_n+u_nq_n}{16}$	=	$\frac{q_nt_n+4r_nt_n+3s^2_n}{8}$

According to our scheme of selection the maximum value of h is $m-1$. The value of h increases from 0 in zygotes which have 2 $(m-1)$ dominants to $m-1$ in zygotes of $m-1$ dominants and then decreases as the number of dominants decreases until it is again 0 in zygotes which have $m-m$ dominants.

In the pentahybrid another factor, which will be treated later, occurs which concerns this "shifting" of gametes in the gamete pairs *rt*, *ss*, and *tr*.

We have now determined which pairs of gametes participate in every particular "shifting" of gametes from generation to generation and also what proportion of the various gametes are produced from each "shift." One more factor must be evaluated before this "shifting," which we will now call "primary shifting" for reasons which will appear later, will be completely solved. As has been stated before, we designated all gametes with an equal number of dominant factors with the same symbol. For example, in the pentahybrid there are 5 different gametes designated by q, 10 designated by r, and 10 designated by s. A given gamete, say q combined with s, would produce a zygote quite different from some other cross of q and s. The only thing all crosses of q and s would have in common would be the total number of dominant (also recessive) factors. They would differ considerably in regard to the number of heterozygous factors. If $10r$ crosses with $10s$ the result will be $100rs$. How many of these 100 combinations will be heterozygous for 1 factor and how many for 3 factors? (Those heterozygous for 5 factors would possess all 5 dominants and would be eliminated). Or, when we cross $10r$ with itself how many combinations are homozygous, and hence are not subject to this gamete "shifting" and how many are heterozygous for 2 factors and are subject to the shifting? (Those heterozygous for 4 factors would contain all m dominants and would be eliminated.) A very simple method of answering this question has been derived empirically. The pentahybrid will be used throughout as an illustration.

First, when any gamete is combined with a gamete which has $m-m$ or 0 dominant factors all of the resultant combinations of gametes are heterozygous for each and every dominant factor which they contain. This gives us for the coefficients of the "shifts" in the last line of our table, all except the first term of the expanded binomial $(1+1)^m$, and gives us u_{n+1} in terms of u_n. (See formula 21.) Thus, in the pentahybrid:

$$u_{n+1}=\frac{5(q_nu_n+4r_nt_n+3_ns_n{}^2)}{8}+\frac{10(r_nu_n+3s_nt_n)}{4}+\frac{10(s_nu_n+t_n{}^2)}{2}+5t_nu_n+u_n{}^2$$

In a like manner the various combinations which contain q can be derived. The coefficients of the zygotes containing q which are allowed to breed according to our scheme of selection (formula 21) are the successive term of $(1+1)^m-(1+1)^{m-1}$ which equals $(1+1)^{m-1}$. Now all pairs must be homozygous recessives in regard to 1 factor (otherwise they would be eliminated), and consequently any gamete so paired with q that the resultant zygote be allowed to reproduce will be heterozygous for *1* less than the number of recessives such a gamete had. This is true of the matings of all of the 5 gametes designated q. Consequently this same "shifting" occurs in all of the gametes labeled q. Thus in the pentahybrid (table 21),

$$q_{n+1}=q_n{}^2+4qr_n+\frac{6qs_nr^2}{2}+\frac{4(qt_n+3rs_n)}{4}+\frac{1(qu_n+4rt_n+3s_n{}^2)}{8}$$

It must be remembered that in our table for squaring the sum of the gametes that each combination of gametes occurs twice, that since $qu=uq$, the number of "shifts" must be the same for both places. We have solved the problem of this shifting for all combinations of q with all other gametes and of n with all other gametes. We may now unite this much of the table for squaring the sum of the gametes. The numbers being only the coefficients of the gamete pairs in which this primary "shifting" takes place.

TABLE 25

Pentahybrid.

$$\begin{array}{rcll}
 & & & \quad q \quad r \quad s \quad t \quad u \\
5\ q_{n+1} & = & 5q & [1+4\ +6\ +4\ +1] \\
10\ r_{n+1} & = & 10r & [2\ \ +\ \ +\ \ +\ \ 1] \\
10\ s_{n+1} & = & 10s & [3\ \ +\ \ +\ \ +\ \ 1] \\
5\ t_{n+1} & = & 5t & [4\ \ +\ \ +\ \ +\ \ 1] \\
u_{n+1} & = & 1u & [5+10+10+5+1]
\end{array}$$

The coefficients in bold face type are those of zygotes homozygous and heterozygous for only 1 factor, consequently no shifting occurs in these cases.

Actual count has shown that,—*the number of gamete pairs in which "shifting" occurs, designated by a given pair of symbols, is, compared with the other pairs which participate in the "shift," proportional to the relative number of such pairs produced by the "shift" in question.* To illustrate: In the "shift" which occurs in the pairs heterozygous for 2 factors (containing $2\ (m-1)-2$ dominant factors) designated qs and r^2, the relation of qs to r^2 is 1 : 1, $(qs+r^2)/2$, consequently there must be as many pairs of r^2 gametes as there are qs gametes. There are in the table 60 gametes designated qs, $(5q\times6s+10s\times3q)$, and consequently there must be 60 r^2 gametes. One of the factors of the 60 gametes is known, that is,

$10r$, (table 25), the other must be $6r$, $(60r^2/10r = 6r)$, and the coefficient of the r^2 pair in the table must be 6 (table 25). Likewise for the "shift" in those pairs heterozygous for 3 factors. Here the relation of qt to rs is $1:3$, $(qt+3rs)/4$, hence there must be three times as many pairs designated rs in which this shift occurs as pairs designated qt. The number of such qt pairs is 40, $(5q \times 4t + 5t \times 4q)$, hence there must be 120 pairs of gametes labeled rs. These fall into 2 equal groups $(rs = sr)$ of 60 each. One factor of each group is known, $10r$ and $10s$, the other factors must consequently be $6s$ and $6r$ (table 25). The coefficients for the pair of gametes heterozygous in regard to 4 factors which participate in this shifting are derived in the same way. Here the proportion of qu to rt to s^2 is $1 : 4 : 3$, $(qu+4rt+3s^2)/8$, hence since there are 10 pairs of qu "shifting" there must be 40 pairs of rt and 30 of sr. Solving for the coefficients in the table as we did for the other 2 "shifts" we get them to be $1qr$, $2rt$, $3s^2$, $4tr$, $5uq$.

Completing the table we get:

TABLE 26

	q	r	s	t	u
$5q_{n+1} = 5q_n$	(1	+ 4	+ 6	+ 4	+ 1)
$10r_{n+1} = 10r_n$	(2	+ 6	+ 6	+ 2	+ 1)
$10S_{n+1} = 10S_n$	(3	+ 6	+ 3	+ 3	+ 1)
$5t_{n+1} = 5t_n$	(4	+ 4	+ 6	+ 4	+ 1)
$u_{n+1} = u_n$	(5	+10	+10	+ 5	+ 1)

An examination of the above table reveals a number of series which can be used as a check upon the accuracy with which the coefficients in question have been derived. If we draw a diagonal line from the upper right hand corner to the lower left the numbers crossed will be 1, 2, 3, 4, ... m. All of the coefficients to the right of and including this line and below it form all except the first term of the expanded binomial $(1+1)^r$, when r equals the number of recessive factors in the gamete of generation G_{n+1} which is being derived from the pairs on that line. Thus then the number of recessives in t_{n+1} is 4, and the coefficients in the next to the last line to the right of and including the diagonal are all except the first term of the expanded binomial $(1+1)^4$. The coefficients to the left of and above this diagonal, also including it, likewise form series. They are the successive terms of the expanded form of $r\ (1+1)^{m-r}$, r being equal to the number of recessives in the gamete in question. Thus the coefficients of the first line are $1\ (1+1)^{m-1}$ of the second line $2\ (1+1)^{m-2}$ of the third line $3\ (1+1)^{m-3}$, etc. There are any number of other series which can also be used as checks. The problem of the effects of this "primary shifting" is thus completely solved.

There now remains only the necessity of combining our various results to enable us to state the value of a gamete in terms of the gametes of the preceding generation. Thus in the case of the tetrahybrid, if there were no "shifting" r_{n+1} would equal $r_n(2q_n+5r+4s_n+t_n)$ but since shifting would occur in 4 of the pairs designated r^2, 2 of those designated rs and 1 of those designated rt, it would be necessary to subtract these from the total number of pairs to derive the number of pairs in which shifting did not occur. By adding the sum of the pairs in which "shifting" does not occur to the sum of the various "shifting" pairs the value of r_{n+1} is obtained thus:

$$r_{n+1}=\underset{(2)}{2r_nq_n}+\underset{(5)}{\left[r_n{}^2+\frac{4(q_ns_n+r_n{}^2)}{2}\right]}+\underset{(4)}{\left[2r_ns_n+\frac{2(q_nt_n+3r_ns_n)}{4}\right]}+\underset{(1)}{\frac{(r_nt_n+s_n{}^2)}{2}}$$

The numbers in parentheses on the lower line are equal to the sum of the coefficients of each of the terms of the above equations and are equal to the coefficients of the terms of the corresponding equations in formula 21.

As we have previously mentioned, another factor enters into the "shifting" of the gamete pairs in the pentahybrid. Some of the pairs, qu, rt, and s^2, are heterozygous for 4 factors. This causes a "primary shifting" which has been solved. In addition certain of these pairs of rt and s^2 are heterozygous for 2 factors, and consequently there enters here into our formulæ a "secondary shifting," the effects of which can be evaluated exactly as those of the "primary shifting."

It is to be noted, first, that all of the zygotes produced by gamete-pairs designated qu are heterozygous for 4 factors and consequently are not affected by this "secondary shift." Second, all of those produced from pairs rt and s^2 which are not heterozygous for 4 factors are heterozygous for *2* except a single pair of those designated s^2, which is homozygous; these, heterozygous for 2 pairs of factors, do participate in the "secondary shift." The number of times this secondary shift occurs can thus be easily obtained by subtracting the exponents of the pairs which undergo the "primary shift" (table 26) formed from the total number of pairs designated by the same symbols (table 15). The result will give the exponents of those which undergo this "secondary shifting." It must be remembered, however, that every time a gamete is paired with those of the group to which it belongs, such combinations being represented by r^2, s^2, t^2, etc., a single combination results which is completely homozygous and hence does not "shift" at all. In the hexahybrid certain of the pairs *ru*, *st*, and *sv* and t^2 experience this "secondary shift," *ru* and *st* being heterozygous for 5 and 3 factors and *sv* and t^2 for 4 and 2. This "second shift" is exactly similar to the first. When the pairs are heterozygous for 2 factors the shift is expressed $(rt+s^2)/2$ or $(sv+t^2)/2$, when they are heterozygous for 3 factors it is expressed $(ru+3st)/4$. To illustrate: the total number of the pair *ru* in the hexahybrid is 6 (table 21). The number of times the pair *ru* participates in the primary shift is 2. Consequently the number which participates in the secondary shift is 4. We have thus derived the coefficient for the first pair of all the groups which participate in the "secondary shifting." If we know this coefficient the other coefficients are easily obtainable, for here as in the primary shifting, *the relative numbers of the pairs which participate in the shift are proportional to the numbers of such pairs produced by the shift in question.* Thus if in the hexahybrid the number of *ru*'s which participate in the shift is 120, $(4u \times 15r + 10r \times 6u)$, the number of *st*'s will be 360, $(ru+3st)/4$ ratio 1 : 3, and thus their coefficients in the table, derived exactly as in the "primary shift" must be 9 and 12. The group of coefficients is then 4 (*ru*), 9 (*st*), 12 (*ts*) and 10 (*ur*).

TABLE 27

Coefficients of the pairs participating in the "secondary shift".

PENTAHYBRID

	q	*r*	*s*	*t*	*u*
5*q*					
10*r*				+3	
10*s*			+6		
5*t*		+6			
u					

HEXAHYBRID

	q	*r*	*s*	*t*	*u*	*v*
6*q*						
15*r*				6	+ 4	
20*s*			9	+ 9	+ 3	
15*t*		6	+12	+ 8		
6*u*		10	+10			
v						

In the heptahybrid still another "shift" appears, a "tertiary shift." It occurs in those gamete groups where a single set of symbols will represent combinations of gametes heterozygous for 6, 4 and 2 factors. Those combinations heterozygous for 6 factors are handled in the "primary shift," those heterozygous for 4 pairs in the "secondary" and those heterozygous for 2 pairs in the "tertiary." Thus in each of the pairs symbolized by *qw*, *rv*, *su* and t^2 there are pairs heterozygous for 6 factors;

in *rv*, *su* and t^2 are those heterozygous for 4 factors; and in *su* and t^2 are those heterozygous for 2 factors. The coefficients of the pairs participating in this "tertiary shift" are found exactly as those for the "secondary." In the third pair of symbols *su* all of the pairs that are not heterozygous for 6 factors and 4 factors are heterozygous for 2 factors (except, of course, a single homozygous combination represented by t^2), and thus participate in this tertiary shift. By adding in the "primary shift" to those of "secondary shift" and subtracting their sum from the total number of such pairs, we get the number which participate in the "tertiary." Knowing the coefficient of the first numbers of a group of pairs participating in a particular shift we can easily obtain the coefficients of the other pairs involved by the method used in the primary and secondary shifts. For the heptahybrid the coefficients of those pairs participating in the tertiary shift are:

TABLE 28

	q	*r*	*s*	*t*	*u*	*v*	*w*
7*q*							
21*r*							
35*s*					+6		
35*t*				+12			
21*u*			+10				
7*v*							
1*w*							

Of course this "tertiary shifting" is present in all hybrids higher than the heptahybrid, and in the nonahybrid a "quarternary shifting" appears. In every higher hybrid where *m* is odd, a new order of shifting appears. However the increasing cumbersomeness of the equations makes it futile to extend this method of calculating the composition of gametes G_{n+1} in terms of those of G_n.

To determine numerical values, since we begin with the complete F_1 heterozygote *AaBbCc* etc., the value of each class of gametes at the commencement is 1: employing successively for one generation at a time the above formula 21, we may determine the proportions for each class of gametes after any number of generations. The proportion of completely dominant zygotes produced from these may then be determined, by the methods to be taken up next.

b. Determination of the proportion of completely dominant zygotes, to be obtained from the gametes of any generation: The total number of zygotes produced by the random mating of any set of gametes is given by squaring the numbers representing the gametes (since each kind of gamete mates in equal numbers with each kind). After we have found the proportions of each type of gamete p, q, r, s, t, etc., for the given generation $(n+1)$, the total number of gametes will be given by an expression made up in accordance with the following:

(1) The class of gametes p, in which all m factors are dominant, does not appear at all.

(2) Of the other classes, containing $m-1, m-2$, etc., dominants, none disappear, and all those containing the same number of dominants are present in equal number.

(3) Hence we have present all possible classes of gametes producible by the polyhybrid of m pairs of allelomorphs, except that containing m dominants. All possible classes are represented, as we have seen, by the expansion of $(1+1)^m$ (see table 12). All possible classes except the first, containing m dominants, will therefore be represented by the expansion of $(1+1)^m$, when this is deprived of its first term. Thus, in the pentahybrid, the gametes will be represented by the expression

$$5q+10r+10s+5t+1u$$

(The diversities in proportions are expressed in the values to be given to q, r, s, etc., not in their coefficients.)

The zygotes of the next generation are produced by the random mating of these; and their number is to be represented by the square of the expression just given. In general, the total number of zygotes of any generation is expressible in terms of the gametes from which they are produced, as follows:

In any polyhybrid of m allelomorphs—

Let p, q, r, s, etc., represent respectively the proportions of the types of gametes containing m, $m-1$, $m-2$, $m-3$, etc., dominant factors.

Let c_1, c_2, c_3, be the respective coefficients of these types of gametes.

Then the total number of zygotes is given by the expression:

$$(c_2q+c_3r+c_4s \cdots \text{to } m \text{ terms})^2 \qquad (29)$$

in which the successive coefficients c_2, c_3, etc., are given by the successive terms, omitting the first of the expansion of $(1+1)^m$.

We require further to know what proportion of these zygotes will be dominant for the entire m pairs of factors (and are hence to be eliminated). We can obtain a general formula for this by induction from the results for the di-, tri-, and tetrahybrid. Brief formulæ for these results are given in formulæ 8, 14, and 17, but for the present purposes it will be necessary to examine in detail the methods by which the formulæ are obtained, and express them in a more general form.

The total number of zygotes of all kinds obtainable from the gametes of a given generation is expressed by squaring the expression for the gametes, as in formula 29. Each kind of gamete is crossed with every other kind, in numbers proportional to the relative numbers of each kind present. To determine which of the resulting combinations will be dominant for all the m factors, the different kinds of gametes must be dealt with individually, since, when a gamete having say two dominant factors is mated with a gamete having say three dominant factors, it depends upon the precise constitution of each whether the results are entirely dominant or not. For example, $Abcd \times aBCD$ gives a completely dominant zygote, while $ABcd \times ABCd$ does not. However, it is sufficient to examine the results for the diverse matings of any typical gamete for any group (as the group r, containing two dominant factors), for any other gametes of that group the results will be the same (when we begin as in the present study, with all kinds of gametes in equal numbers).

Proceeding to such an examination, it is to be recalled that the gamete p (containing m dominant factors) does not appear (after G_1). For the dihybrid there remains in any generation only the set of gametes represented by $2q+r$. Mating each of these with each other, we find that completely dominant zygotes (containing AB) are given simply by $2q^2$, which may be written for our purposes

$$2q\,(q)$$

In the trihybrid, the gametes of which are represented in any generation by $3q+3r+s$, matings show that completely dominant zygotes are given by the following:

$$\begin{array}{l} 3q\ (2q+r) \\ 3r\ (q) \end{array}$$

And in the tetrahybrid completely dominant zygotes are produced by the mating of gametes which we represent as follows:

$$\begin{array}{l} 4q\ (3q+3+s) \\ 6r\ (2q+r) \\ 4s\ (q) \end{array}$$

The law of formation of these expressions is obvious on inspection, so that one can readily write them out for higher polyhybrids. The pentahybrid, for example, evidently yields for the completely dominant zygotes:

$$\begin{array}{l} 5q\ (4q+6r+4s+t) \\ +10q\ (3q+3r+s) \\ +10r\ (2q+r) \\ +\ 5s\ (q) \end{array}$$

For a hybrid of m allelomorphs, the sum of the completely dominant zygotes is given by the sum of $m-1$ expressions, each of which consists of a quantity outside the parenthesis and a quantity within it. The $m-1$ quantities outside the parenthesis are successively c_2q, c_3r, c_4s, etc., in which the coefficients c_2, c_3, etc., are respectively the second, third, fourth, and succeeding terms of the expansion of $(1+1)^m$. The quantities within the parentheses are c_2q c_3r c_4s, and so on, the coefficients for the first parenthesis being given by all but the first term of $(1+1)^{m-1}$, those for the second term by all but the first term of $(1+1)^{m-2}$, and so on, the coefficient of the last parenthesis being given by $(1+1)^0$, so that it is 1.

Since the k term of $(1+1)^m$ is $m!/(k-1\ !m-(k-1)!)$, we can write a general formula for the sum of the completely dominant zygotes, in terms of the gametes from which they are derived. This divided by the expression given in formula 29 will give the actual proportion in any generation that the completely dominant zygotes are of the whole. This formula for the proportions is as follows:

FORMULA 30

Proportions of the entire set of zygotes that are completely dominant, in any generation, in terms of the gametes from which they are derived, for any polyhybrid of m pairs of allelomorphs, when the completely dominant zygotes have been eliminated throughout the preceding generations. The numerator is the sum of m − 1 expressions, constituted as follows:

$$\frac{m}{1!m-1!}q\frac{m-1!}{1!m-2!}q\frac{m-1!}{2!m-3!}p\frac{m-1!}{3!m-4!}s+\cdots \text{ to } m-1 \text{ terms}$$

$$\frac{m}{2!m-2!}p\frac{m-2!}{1!m-3!}q\frac{m-2!}{2!m-4!}r+\cdots \text{ to } m-2 \text{ terms}$$

$$\frac{m!}{3!m-3!}s\frac{m-3!}{1!m-4!}q\frac{m-3!}{2!m-5!}r+\cdots \text{ to } m-3 \text{ terms}$$

and so on until there are $m-1$ such expressions.

The denominator is:

$$(c_2q+c_3r+c_4s\ \cdots,\ \text{to } m \text{ terms})^2,$$

in which the successive coefficients c_2, c_3, c_4, etc., are given by the successive terms, *omitting the first, or* c_1, of the expansion of $(1+1)^m$.

Thus to determine for any polyhybrid of m pairs of allelomorphs the proportion of completely dominant zygotes present in the successive generations, when all these are eliminated from each generation as they appear, one begins with each of the $m-1$ classes of gametes o, p, q, r, s, t, etc., of G_1 in the proportion 1. These are then transformed into the gametic proportions of the succeeding generations by the method given.

Then for each generation the proportion of completely dominant zygotes is determined by the use of formula 30. Curves for the various polyhybrids up to $m=4$, thus determined, are given in Part V of this paper.

PART II.

THE ELIMINATION IN EACH GENERATION OF ALL INDIVIDUALS WHO ARE NOT DOMINANT FOR ALL OF THE m INDEPENDENT FACTORS IN A POLYHYBRID OF m ALLELOMORPHS

This problem is the complement of the one treated in Part I. If, in Part I, we were eliminating a character due to the simultaneous presence of m dominant genes, we are here breeding for that character and selecting out all individuals who do not possess it, that is, all individuals who show any recessive traits. The method of deriving the equations, which show the effect of successive elimination each generation of all undesirable characters, is the same one used in Part I. Equations are derived for the simpler hybrids and, from them are obtained the underlying fundamental series which make it possible through mathematical induction, to obtain equations for all hybrids.

As we are here selecting out the recessives we may use the monohybrid as the simplest case. We could not do this when we were eliminating dominants, for the single elimination of a dominant from a monohybrid leaves the strain homozygous-recessive and the curve of the appearance of dominants reaches zero in the F_3 generation. The recessive, however, cannot be eliminated by any finite number of selections, as was shown by JENNINGS (1916), who derived a formula for its occurrence when it is not allowed to reproduce. The formula is

$$\frac{1}{(n+2)^2}$$

where n equals the number of selections. In the present paper n is used as the number of the filial generation, beginning with the parental cross AA by aa, and with this value of n the formula would be

$$\frac{1}{n^2}$$

This formula is the starting point of a series which solve all cases. It was possible to carry the formulæ much further than those derived in Part I. Instead of having to derive the gametes of a given generation in terms of the gamete of the preceding generation and repeating the performance for each generation until the desired generation is reached, we can here reduce the formulæ to two factors, *m*, the number of allelomorphs, and *n* the number of the filial generation.

1. *The dihybrid*

The problem of the results of eliminating in each generation all of the individuals of a dihybrid stock who have one or more recessive traits, is attached by first deriving a method for expressing the gametes of the $n+1$ generation (G_{n+1}) in terms of the gametes of the *n*th generation (G_n). The necessary formulæ can be derived from tables 1 and 8. They are:

$$p_{n+1} = {}_n{}^2p + 2p_nq_n + \frac{p_nr_n + q_n{}^2}{2}$$

$$q_{n+1} = p_nr_n + \frac{p_nr_n + q_n{}^2}{2}$$

$$r_{n+1} = \frac{p_nr_n + q_n{}^2}{2}$$

Starting in the G_1 generation with *p*, *q*, and *r* each equalling 1, we get upon simpification:

TABLE 31

	G_1	G_2	G_3	G_4	G_5	G_6	G_n
$p =$	1	4	9	16	25	36	n^2
$q =$	1	2	6	4	5	6	n^1
$r =$	1	1	1	1	1	1	n^0 or 1

The formula for deriving the percentage of zygotes containing some recessive characters from a given set of gametes is: (see tables 8 and 30)

$$\frac{2q^2 + 4qr + r^2}{(p + 2q + r)^2}$$

Substituting in the above for *p*, *q*, and *r* their values in terms of any given generation G_n, $p = n^2$, $q = n$, $r = n$ or 1, we get

$$= \frac{2n^2 + 4_n + 1}{(n+1)^4}$$

Simplifying

$$=\frac{2(n^2+2n+1)-1}{(n+1)^4}$$

$$=\frac{2(n+1)^2}{(n+1)^4}-\frac{1}{(n+1)^4}$$

$$=\frac{2}{(n+1)^2}-\frac{1}{(n+1)^4}$$

Now expressing our formula, at present in terms of the gametes of G_n generation, in terms of F_n (G_n producing F_{n+1}), we get for the proportion of zygotes in any generation that are not completely dominant (and are hence to be eliminated from the parents of the next generation)

$$\frac{2}{n^2}-\frac{1}{n^4}$$

As n increases, the value of the second term of our formula so decreases in proportion to the first term that it can be omitted without altering to any appreciable extent the value of the formula when n is large. When $n=10$ the value of the second term is only 1/2 percent of the first.

2. *The trihybrid*

The problem of the results of eliminating from a trihybrid stock all individuals who are not dominant for all three allelomorphs is attacked precisely as in the case of the dihybrid. The formulæ for expressing the gametes of the $n+1$ generation in terms of those of the nth generation are:

$$p_{n+1}=p_n^2+3p_nq_n+3\frac{p_nr_n+q_n^2}{2}+\frac{p_ns_n+3q_nr_n}{4}$$

$$q_{n+1}=p_nq_n+2\frac{p_nr_n+q_n^2}{4}+\frac{p_ns_n+3q_nr_n}{4}$$

$$r_{n+1}=\frac{p_nr_n+q_n^2}{2}+\frac{p_ns_n+3q_nr_n}{4}$$

$$s_{n+1}=\frac{p_ns_n+3q_nr_n}{4}$$

As p, q, r, and s equal 1 in the G_1 generation, substituting in the formulæ we get their numerical values to be:

	G_1	G_2	G_3	G_4	G_n
p_1	1	8	27	64	n^3
q_1	1	4	9	16	n^2
r_1	1	2	3	4	n^1
s_1	1	1	1	1	n^0 or 1

The corresponding formulæ for deriving the percentage of individuals to be eliminated from a given group of gametes is:

$$\frac{3q(q+4r+2s)+3r(3r+2s)+s^2}{(p+3q+3r+s)^2}$$

Substituting for p, q, r, and s in value in terms of n, the formula is

$$=\frac{3n^4+12n^3+15n^2+6n+1}{(n+1)^6}$$

Simplifying

$$=\frac{3(n+1)^4}{(n+1)^6}-\frac{3n^2+6n+2}{(n+1)^6}$$

$$=\frac{3}{(n+1)^2}-\frac{3(n+1)^2}{(n+1)^6}+\frac{1}{(n-1)^6}$$

$$=\frac{3}{(n-1)^2}-\frac{3}{(n+1)^2}+\frac{1}{(n+1)^6}$$

Expressing the above in terms of F_n, we get the proportion of zygotes still containing some recessive characters in the nth generation,

$$\frac{3}{n^2}-\frac{3}{n^4}+\frac{1}{n^6}$$

3. *The tetrahybrid*

The results of eliminating any and all recessives from a polyhybrid stock are obtained exactly as in the di- and trihybrids. The G_{n+1} gametes expressed in terms of G_n gametes are

$$p_{n+1}=p_n{}^2+4p_nq_n+6\frac{p_nr_n+q_n{}^2}{2}+4\frac{p_ns_n+3q_nr_n}{4}+\frac{p_nt_n+4q_ns_n+3r^2{}_n}{8}$$

$$q_{n+1}=p_nq_n+3\frac{p_nr_n+q_n{}^2}{2}+4\frac{p_ns_n+3q_nr_n}{4}+\frac{p_nt_n+4q_ns_n+3r_n{}^2}{8}$$

$$r_{n+1}=\frac{p_nr_n+q_n{}^2}{2}+2\frac{p_ns_n+3q_nr_n}{4}+\frac{p_nt_n+4q_ns_n+3r_n{}^2}{8}$$

$$s_{n+1}=\frac{p_ns_n+3q_nr_n}{4}+\frac{p_nt_n+4q_n+3r_n{}^2}{8}$$

$$t_{n+1}=\frac{p_nt_n+4q_ns_n+3r_n{}^2}{8}$$

The numerical values of the gametes, derived as in the di- and trihybrids are:

TABLE 32

	G_1	G_2	G_3	G_4	G_n
$p=$	1	16	81	256	n^4
$q=$	1	8	27	64	n^3
$r=$	1	4	9	16	n^2
$s=$	1	2	3	4	n^1
$t=$	1	1	1	1	n^0

The formula for obtaining the percentage of zygotes which have some recessive characters is:

$$\frac{4q(q+6r+6s+2t)+6r(5r+8s+2t)+8s(2s+t)+t^2}{(p+4q+6r+4s+t)^2}$$

Expressed in gametes in terms of generation G_n:

$$\frac{4n^6+24n^5+54n^4+56n^3+28n^2+8n+1}{(n+1)^8}$$

Simplifying:

$$=\frac{4(n+1)^6}{(n+1)^8}-\frac{6n^4+24n^3+32n^2+16n+3}{(n+1)^8}$$

$$=\frac{4}{(n+1)^2}-\frac{6(n+1)^4}{(n+1)^8}+\frac{4n^2+8n-3}{(n+1)^8}$$

$$=\frac{4}{(n+1)^2}-\frac{6}{(n+1)^4}+\frac{4(n+1)^2}{(n+1)^8}-\frac{1}{(n+1)^8}$$

$$=\frac{4}{(n+1)^2}-\frac{6}{(n+1)^4}+\frac{4}{(n+1)^6}-\frac{1}{(n+1)^8}$$

Translating from G_n to terms of F_n generation:

$$\frac{4}{n^2}-\frac{6}{n^4}+\frac{4}{n^6}-\frac{1}{n^8}$$

4. *The polyhybrid*

We are now in a position to derive formulæ for solving the problem of the effect of eliminating all individuals who are not completely dominant from any polyhybrid strain. Let us repeat the formulæ just derived; which show the percentage of individuals who have some recessive characters in mono-, di-, tri-, and tetrahybrids;

$$\text{Monohybrid} \quad \frac{1}{n^2}$$

$$\text{Dihybrid}\quad \frac{2}{n^2}-\frac{1}{n^4}$$

$$\text{Trihybrid}\quad \frac{3}{n^2}-\frac{3}{n^4}+\frac{1}{n^6}$$

$$\text{Tetrahybrid}\quad \frac{4}{n^2}-\frac{6}{n^4}+\frac{4}{n^6}-\frac{1}{n^8}$$

The formulæ are all series of fractions with signs alternating plus and minus. The numerators in every case are given by all except the first term of the expanded binomial $-(1-1)^m$. The first term in each of the denominators is n^2 until the last term is reached which is n^{2m}.

Let $c_1, c_2, c_3, \cdots c_{m+1}$ be the successive terms of expanded binomial $(1+1)^m$. Then the formula for the elimination of all individuals not completely dominant from a polyhybrid stock would be:

$$\frac{c_2}{n^2}-\frac{c_3}{n^4}+\frac{c_4}{n^6}-\frac{c_5}{n^8}\cdots\pm\frac{1}{n^{2m}}$$

Or if we wish to express this in terms of $m!$:

$$\frac{m!}{1!m-1!n^2}-\frac{m!}{2!m-2!n^4}+\frac{m!}{3!m-3!n^6}-\cdots\pm\frac{m!}{m!0!n^{2m}}$$

As n increases, the value of the latter terms of the formula so decrease in proportion to the first terms that they can be ignored without appreciably altering the value of the formula as a whole. Taking into consideration only the first two terms, the formula would read:

$$\frac{m}{n^2}-\frac{m(m+1)}{2n^4}$$

A further simplification would give the approximate formula:

$$\frac{m}{n^2}$$

which as n increased would approach extremely close to the real value of the entire formula.

To illustrate: In an octahybrid stock the percentage of individuals who possessed some recessive trait in the F_{20} generation is:

$$=\frac{8}{20^2}-\frac{28}{20^4}+\frac{56}{20^6}-\frac{70}{20^8}+\frac{56}{20^{10}}-\frac{28}{20^{12}}+\frac{8}{20^{14}}-\frac{1}{20^{16}}$$

$$=1.9825 \text{ percent}$$

Or, if we use only the first term:

2 percent

PART III

THE ELIMINATION IN EACH GENERATION OF ALL INDIVIDUALS RECESSIVE FOR ALL *m* INDEPENDENT FACTORS IN A POLYHYBRID OF *m* ALLELOMORPHS

1. *Method of deriving zygotes of generation* F_n *from gametes of generation* G_n

The problem presented in this section is attacked in the same manner as those in Parts I and II. In only the simplest case, that of eliminating the recessives from a monohybrid, (solved by JENNINGS (1916) whose equation, $1/(n+2)^2$, is modified to $1/n^2$ by changing the value of n from the number of selections to the number of the filial generation (F_n)[1] has a formula been derived for expressing the effects of this type of selection in terms of n. The formulas for the polyhybrids are for calculating the gametes of generation G_{n+1} in terms of generation G_n and for determining the proportion of completely recessive individuals from any set of gametes. The method of deriving these formula is the same as that used in Part I.

If we group together all gametes which have the same number of dominant factors we will have $m+1$ such groups and, if the groups are arranged in order according to the number of dominant factors they contain beginning with the one which has all m dominant factors and ending with one which is completely recessive, the number of different gametes in each successive group will be equal to the successive terms of the expanded binomial $(1+1)^m$. The zygotes of any generation F_n are obtained by crossing the gametes of G_n in all possible ways. This is best accomplished by squaring their sum. The completely recessive zygotes are produced only by a combination of gametes neither of which contains any dominant factors. According to our system of mating these homozygous recessive zygotes are obtained by squaring the completely recessive gametes. To obtain then the percentage of completely recessive individuals in the general population it is only necessary to divide the square of the completely recessive gamete by the square of the sum of all of the gametes which go to make up the population in question. Using the symbols p, q, r, s, etc., as in Parts I and II, we get the proportion of completely recessive individuals in the generation F_n in terms of the gametes of G_n expressed by the following formulæ:

$$\text{for the monohybrid} = \frac{q^2}{(p+q)^2}$$

---

[1] This formula, which forms the starting point for the series here treated, is the same one which formed the starting point for the series dealt with in Part II, for in the monohybrid these two schemes of selection would eliminate the same individual.

$$\text{for the dihybrid} = \frac{r}{(p+2q+r)^2}$$

$$\text{for the trihybrid} = \frac{s^2}{(p+3q+3r+s)^2}$$

$$\text{for the tetrahybrid} = \frac{t^2}{(p+4q+6r+4s+t)^2}$$

and so on.

2. *Method of deriving gametes of generation n+1 from gametes of generation* G_n

To obtain the gametes in generation G_{n+1} in terms of those of G_n we will proceed as in Part I and treat the gametes first as if they were immutable, that is, as if a zygote could produce only the gametes out of which it itself was formed. To repeat, this assumption is true when the zygote is homozygous or heterozygous for a single factor; it is not the case when the zygote is heterozygous for two or more factors.

Now the relative number of any gamete in generation G_{n+1} can be easily calculated if the number of zygotes in generation F_n, which produce it, is known. According to our postulate, a gamete in generation G_{n+1} would be produced from those zygotes in generation F_n into whose formation went that same gamete in generation G_n. The number of these zygotes would be equal to the product of the gamete in question times the sum of the gametes. Thus in the case of the hybrid:

$$p_{n+1} = p_n(p_n + 2q_n + r_n)$$

in the trihybrid:

$$p_{n+1} = p_n(p_n + 3q + 3r_n + s_n)$$

in the tetrahybrid:

$$p_{n+1} = p_n(p_n + 4q_n + 6r_n + 4s_n + \mathrm{t}_n)$$

Our scheme of selection eliminates but a single combination of gametes, r^2 in the dihybrid, s^2 in the trihybrid, t^2 in the tetrahybrid, etc. We can now write the complete formula, using the tetrahybrid as an example.

TABLE 33

$$\begin{aligned}
p_{n+1} &= p_n\ (p_n+4q_n+6r_n+4s_n+t_n)\\
q_{n+1} &= q_n\ (p_n+4q_n+6r_n+4s_n+t_n)\\
r_{n+1} &= r_n\ (p_n+4q_n+6r_n+4s_n+t_n)\\
s_{n+1} &= s_n\ (p_n+4q_n+6r_n+4s_n+t_n)\\
t_{n+1} &= t_n\ (p_n+4q_n+6r_n+4s_n)
\end{aligned}$$

It would be well to record here several interesting facts about the above formula. It would hold for all cases but for that "shifting" of gametes which occurs when the zygotes are heterozygous for two or more factors, which was discussed in Part I. This "shifting" does not occur at all in the monohybrid and in the polyhybrid does not affect any generation prior to F_4. This formula can consequently be used for the calculation of the gametes in any generation of the monohybrid and in generation G_2 and G_3 of the polyhybrid in terms of the gametes of the preceding generation. Combined with the formula for calculating the percentage of completely recessive individuals from any group of gametes, it can be stated in terms of m and n. Thus:

$$\frac{1}{(n-2+2^m)^2}$$

It can be seen that the formula, $1/n^2$, for calculating the percentage of homozygous recessive individuals in the monohybrid when none are allowed to reproduce is but a special case of the above.

The "shifting" of gametes which occurs when the population is subjected to the type of selection we are now considering is essentially the same as that which occurs in Part I, and can best be described by a particular example. If we draw a diagonal line on table 33 from the upper right-hand corner at p_nt_n to the lower left hand corner at p_nt_n this line will cross all of the pairs which participate in a single shift, that is, pt, qs, rr, sq, and tp, lines drawn parallel with this diagonal from p_ns_n and q_nt_n respectively will each cross a group of the pairs which participate in another shift, that is, ps, qr and qt, rs. Two more parallel lines from pair p_nr_n and pair t_nr_n will cross the pairs which participate in the remaining two "shifts," that is, pr, qq, and rt ss. The number of times a particular combination of gametes participates within a shift is determined exactly the same as in Part I. It is necessary to give here only the results. Thus the effect of the single "shift" which occurs in the dihybrid is stated:

$$\frac{p_nr_n+q_n^2}{1}$$

In the trihybrid there are three shifts:

$$\frac{p_nr_n+q_n^2}{2},$$

$$\frac{p_ns_n+3q_nr_n}{4} \text{ and } \frac{q_ns_n+r_n^2}{2}$$

In the tetrahybrid the shifts are:

$$\frac{p_nr_n+q_n^2}{2},\ \frac{p_ns_n+3q_nr_n}{4},\ \frac{p_nt_n+4q_ns_n+3r_n^2}{8},\ \frac{q_nt_n+3r_ns_n}{4},\ \frac{r_nt_n+s_n^2}{2}$$

In addition there is the single secondary shift $(q_ns_n+r^2{}_n)/2$.

These above stated "shifts" are substituted for gamete pairs exactly as in Part I though they have quite different coefficients. The following table, homologous to table 26, Part I, gives the coefficients of the primary "shifts" as they occur in the tetrahybrid.

TABLE 34

G_{n+1}		G_n	p	q	r	s	t
p_{n+1}	=	p_n	(1	+ 4	+ 6	+ 4	+ 1)
$4q_{n+1}$	=	$4q_n$	(1	+ 3	+ 3	+ 1	+ 1)
$6r_{n+1}$	=	$6r_n$	(1	+ 2	+ 1	+ 2	+ 1)
$4s_{n+1}$	=	$4s_n$	(1	+ 1	+ 3	+ 3	+ 1)
t_{n+1}	=	t^n	(1	+ 4	+ 6	+ 4)	

The coefficients in the above table can be expressed in a general form which will hold for all polyhybrids. Let d equal the number of dominant factors and r the number of recessive which the gamete has whose value in generation G_{n+1} is to be obtained. Then the coefficients of the shifts which are to replace the combinations in table 33 are the successive terms of the expanded binomial $(1+1)^d$ plus all except the first term of the expanded $(1+1)^r$. The combination t_n^2 is of course eliminated. By combining table 33 and 34 as the two like tables were combined in Part I, we can get the complete formulæ for obtaining gametes of generation G_{n+1} in terms of G_n. The secondary shift which appears in the tetrahybrid is treated exactly as it is in Part I. The formulæ for obtaining G_{n+1} from G_n in the dihybrid and trihybrid:

Dihybrid

$$p_{n+1}=p_n^2+2p_nq_n+\frac{p_nr_n+q^2{}_n}{2}$$

$$q_{n+1}=p_nq_n+\frac{p_nr_n+q_n^2}{2}+q_n^2+q_nr_n$$

$$r_{n+1}=\frac{p_nr_n+q_n^2}{2}+2q_nr_n$$

Trihybrid

$$p_{n+1}=p_n^2+3p_nq_n+3\frac{p_nr_n+q_n^2}{2}+\frac{p_ns_n+3q_nr_n}{4}$$

$$q_{n+1}=p_nq_n+q_n^2+2\frac{p_nr_n+q_n^2}{2}+2q_nr_n+\frac{p_ns_n+3q_nr_n}{4}+\frac{q_ns_n+r_n^2}{2}$$

$$r_{n+1}=\frac{p_nr_n+q_n^2}{2}+2q_nr_n+\frac{p_ns_n+3q_nr_n}{4}+r_n^2+2\frac{q_ns_n+r_n^2}{2}+r_ns_n$$

$$s_{n+1}=\frac{p_ns_n+3q_nr_n}{4}+3\frac{q_ns_n+r_n^2}{2}+3r_ns_n$$

It would, of course, be desirable to obtain a formula which would allow us to calculate the percentage of completely recessive individuals appearing in any generation, F_n, without obtaining it for all preceding generations, under conditions in which none such are allowed to reproduce. Such a formula of course exists in the case of the monohybrid. No such exact formula has been derived for the polyhybrids. However it has been found that the percentage of such individuals which occur in those generations of the dihybrid which have been accurately worked out, when $n>7$, is very closely approximated by the formula:

$$\frac{\sqrt{n+2}-2.05}{n^2}$$

(see table 36.)

A formula in terms of n which crudely shows the effects of selection upon the trihybrid is:

$$\frac{4}{(\sqrt{8n-15}+15)^2}$$

PART IV.

THE ELIMINATION IN EACH GENERATION OF ALL INDIVIDUALS (A) CONTAINING TWO OR MORE INDEPENDENT DOMINANT FACTORS, AND (B) CONTAINING THREE OR MORE INDEPENDENT DOMINANT FACTORS IN A POLYHYBRID OF *m* ALLELOMORPHS

The problems in Parts I to III dealt with the results either of eliminating a certain completely defined combination or of retaining a certain completely defined combination. To illustrate with the pentahybrid:

Part I eliminates only the combination *ABCDE*

Part II retains only the combination *ABCDE*

Part III eliminates only the combination *aabbccddee*. (Retention of only this last combination, of course, leaves the stock at once uniformly pure recessive.)

The present Part IV deals with a case differing from those of the past three parts, in that any one of several combinations is eliminated and any one of several combinations is retained. Thus, when all individuals containing any two or more dominants are eliminated, there are retained (1) all pure recessives, and (2) all that are dominant only in any one character. It appears desirable to deal briefly with these cases, as steps toward a general solution of the problem of eliminating individuals of any description from a polyhybrid.

It is quite possible that special ability in the human stock is determined by some such combination as we are now eliminating. It is obvious that all ability is not alike and consequently not determined by the same combination of genes though it may well be that different types of ability have some genes in common.

The generalized problem is: *Results of eliminating from a hybrid of m allelomorphs all individuals possessing any k or more dominant characters. Only the cases where k is 2 and where k is 3 are dealt with in the present paper.*

The formulæ here derived are for calculating the value of each kind of gamete in terms of the gametes of the preceding generation and for obtaining any given set of gametes from the percentage of zygotes to be eliminated. The formulæ are derived exactly as are those used in Part I, and can be obtained from tables 1 and 3. We shall derive formulæ for these different values of m—when $m=2$, $m=3$, and $m=4$—and from these further derive a generalized formula for any value of m. As the method of deriving the three sets of formulæ has already been explained (Part I) we will here merely give the formulæ in question.

1. The formulæ for showing the results of eliminating from a dihybrid stock any combination of two dominant factors has been derived in Part I. It is repeated here as it is the first of the series. The gametes of generation G_{n+1} in terms of the gametes of generation G_n are:

$$q_{n+1}=q_n(q_n+r_n)$$
$$r_{n+1}=r_n(2q_n+r_n)$$

noting that in this and all other cases here discussed, in generation G_1 all gametes are equal to unity.

The percentage of zygotes containing 2 dominant factors in terms of any set of gametes is:

$$\frac{2q^2}{(2q+r)^2}$$

2. When we eliminate each generation from a trihybrid all individuals who possess 2 or more dominant factors, the gametes of generation G_{n+1} in terms of the gametes of generation G_n are:

$$q_{n+1}=q_n(q_n+r_n)$$
$$r_{n+1}=r_n(3q_n+r_n)$$

The formula for calculating the percentage of zygotes eliminated in each generation in terms of these gametes is:

$$\frac{6q^2}{(3q+r)^2}$$

3. When $m=4$ the corresponding equations are for the gametes:

$$q_{n+1}=q_n(q_n+r_n)$$
$$r_{n+1}=r_n(4q_n+r_n)$$

and for the zygotes eliminated:

$$\frac{12q^2}{(4q+r)^2}$$

To restate the above equations in a generalized form where m may have any value: When any combination of 2 or more dominant factors is eliminated from a polyhybrid stock, the value of the gametes of any generation in terms of the gametes of the preceding generation is:

$$q_{n+1}=q_n(q_n+r_n)$$
$$r_{n+1}=r_n(mq_n+r_n)$$

The formula for deriving the percentage of zygotes containing 2 or more dominant factors from the above gametes is:

$$\frac{m(m-1)q^2}{(mq+r)^2}$$

To illustrate: The formulæ for showing the effects of eliminating any combination of 2 or more dominants from an octahybrid are (substituting in the above) for the gametes:

$$q_{n+1}=q_n(q_n+r_n)$$
$$r_{n+1}=r_n(8q_n+r_n)$$

and for the zygotes:

$$\frac{56q^2}{(8q+r)^2}$$

The effects of the aforementioned type of selection on various polyhybrid stocks are shown in table 37. It will be seen from this table that the individuals containing any combination of 2 or more dominant factors are eliminated more rapidly from a stock as m increases. Thus these individuals disappear more slowly in a dihybrid than in any other stock.

B. *Results of eliminating in each generation all of the individuals, in a polyhybrid stock, that possess 3 or more dominant factors:* This problem is in all essentials identical with the one handled in A. We assign here, just as we did in A, three specific values to m ($m=3$, $m=4$, and $m=5$) and generalize our resulting formulæ into such terms that m may have any value.

We derive the gametes of generation G_{n+1} in terms of those of generation G_n exactly as in Part I, and calculate the percentage of zygotes eliminated each generation in terms of any given set of gametes.

1. The first case, that of the trihybrid ($m=3$) has been solved in Part I. When we eliminate from a trihybrid in each generation all individuals who possess 3 or more dominant factors, the gametes of generation G_{n+1} in terms of those of G_n are:

$$q_{n+1}=q_n{}^2+2q_nr_n+\frac{q_ns_n+r_n{}^2}{2}$$

$$r_{n+1}=2q_nr_n+2\frac{q_ns_n+r_n{}^2}{2}+r_ns_n$$

$$s_{n+1}=3\frac{q_ns_n+r_n{}^2}{2}+3r_ns_n+s_n{}^2$$

The percentage of zygotes eliminated each generation, in terms of the above, is:

$$\frac{6q(q+r)}{(3q+3r+s)^2}$$

When $m=4$ (the tetrahybrid) the corresponding formulæ are for the gametes:

$$q_{n+1}=q_n{}^2+2q_nr_n+\frac{q_ns_n+r_n{}^2}{2}$$

$$r_{n+1}=3q_nr_n+3\frac{q_ns_n+r_n{}^2}{2}+r_n{}^2+r_ns_n$$

$$s_{n+1}=\frac{q_ns_n+r_n{}^2}{2}+4r_ns_n+s_n{}^2$$

and for the zygotes:

$$\frac{6q(5q+4r)}{(6q+4r+s)^2}$$

3. When $m=5$ (the pentahybrid) the corresponding formulæ are for the gametes:

$$q_{n+1}=q_n^2+2q_nr_n+\frac{q_ns_n+r_n^2}{2}$$

$$r_{n+1}=4q_nr_n+4\frac{q_ns_n+r_n^2}{2}+r_n^2+r_ns_n$$

$$s_{n+1}=10\frac{q_ns_n+r_n^2}{2}+5r_ns_n+s_n^2$$

and for the zygotes:

$$\frac{30q\quad(3q\quad 2r)}{(10q+5r+s)^2}$$

4. The above series of equations derived for 3 specific values of m can be expressed in a general form. Let $c_1, c_2, c_3 \cdots\cdots c_{n+1}$ be the successive terms of the expanded binomial $(1+1)^m$. Then, when any combination of three or more dominants is eliminated from a polyhybrid stock of m allelomorphs, the gametes of generation G_{n+1} in terms of the preceding generation G_n are:

$$q_{n+1}=q_n^2+2q_nr_n+\frac{q_ns_n+r_n^2}{2}$$

$$r_{n+1}=(c_2-1)q_nr_n+(c_2-1)\frac{q_ns_n+r_n^2}{2}+r_ns_n$$

$$s_{n+1}=c_3\frac{q_ns_n+r_n^2}{2}+c_2r_ns_n+s_n^2$$

The percentage of zygotes eliminated in each generation in terms of these gametes is:

$$\frac{q\{[c_3(c_3-1)q+2[c_2c_3-c_2(c_2-1)]\}r}{(c_3q+c_2r+s)^2}$$

Illustrating with an octahybrid: If we let $m=8$, the corresponding formulæ are for the gametes:

$$q_{n+1}=q_n^2+2q_nr_n+\frac{q_ns_n+r_n^2}{2}$$

$$r_{n+1}=7q_nr_n+r_n^2+7\frac{q_ns_n+r_n^2}{2}+r_ns_n$$

$$s_{n+1}=28\frac{q_ns_n+r_n^2}{2}+8r_ns_n+s_n^2$$

and for the zygotes derived from them:

$$\frac{84q(9q+4r)}{(28q+8r+s)^2}$$

GENERAL SUMMARY

It is obvious that before any of the results here obtained can be applied to a human population many more factors which enter into the problem of the effects of selection, must be evaluated. In every case here treated fortuitous mating was assumed, while in a human population it can scarcely be doubted that selective mating has a great influence upon the distribution and combination of those genes whose presence in an individual is a prerequisite for any special achievement. Linkage between certain of the genes concerned would also affect the results of selection—at least in the earlier generations. The present work has dealt with only certain of those effects of selection where there was a complete elimination of a certain type while in a human population such a complete elimination of a type must be quite rare. The relationship, however, between the number of genes which determine a given type and the recurrence of that type in a population in which it does not reproduce itself is a most important one and an evaluation of it is necessary to any understanding of the effects of selection. In a certain specific case, whose description follows, this has been done.

When all individuals dominant for all m factors in a polyhybrid of m allelomorphs are eliminated in the F_2 generation and the remainder of the population bred at random, there is a sudden sharp drop in the percentage of such individuals who appear in the F_3 generation. The results of a continued elimination of such individuals when plotted form a smooth curve which approaches zero as the number of such selections becomes infinite (figure 1). The more factors involved in the type that is eliminated (the greater the value of m), the slower the type is eliminated by selection. As we arbitrarily start with a population completely heterozygous in the F_1 generation and begin the selection process with generation F_2, the greater the value of m the fewer the individuals we have to eliminate at the start. Thus the curve showing the rate of their disappearance begins at a lower point. A consequence of this and the slower disappearance of types determined by many genes is that each of these curves intersects all the others. A single selection (F_3) will bring the percentage of completely dominant individuals in a dihybrid stock below that of like individuals in a trihybrid and two selections (F_4) below those in a tetrahybrid. The trihybrid curve falls below that of the tetrahybrid in generation F_7. The ratios of the rates of disappearance of the completely dominant individuals in the various polyhybrid stocks are somewhat greater than the inverse ratios of the number of factors involved. Thus at the F_{20} generation the percentage of completely dominant individuals in the dihybrid is less than two-thirds of those in the trihybrid which in turn is less than three-fourths of those in the tetrahybrid (table 35).

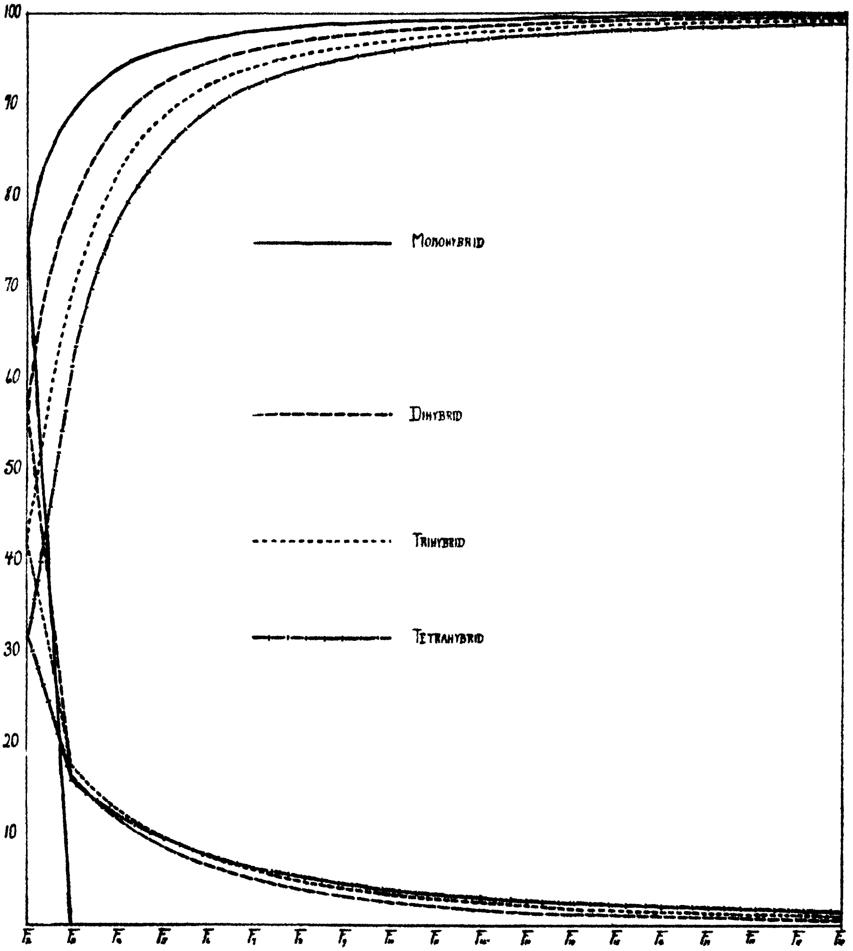

FIGURE 1.—The straight lines show the percentage of individuals dominant for all *m* factors in a mono-, di-, tri-, and tetrahybrid when there is no selection. The upper curves show the percentage of such individuals appearing each generation when they alone are allowed to breed. The lower curves show the percentage of such individuals appearing in the stock when they are not allowed to breed and the rest of the stock bred at random.

TABLE 35

Tables showing precentage of completely dominant individuals appearing each generation in several polyhybrid stocks, under conditions in which no completely dominant individual is allowed to breed.

	$\dfrac{2}{(n-\sqrt{\frac{n}{2}}+1)^2}$	DIHYBRID	TRIHYBRID	TETRAHYBRID
F_2	.500000	.5625	.4219	.3164
3		.1632	.1753	.1578
4	.1555	.1189	.1282	.1217
5		.0876	.0983	.09460
6	.0720	.0658	.07658	.07590
7		.0505	.06153	.06248
8	.0408	.0397	.04924	.05236
9		.0309	.04176	.04453
10	.0260	.0260	.03523	.03836
11		.0215	.03010	.03341
12	.01796	.01807	.02602	.02936
13		.01534	.02272	.02603
14	.01310	.01317	.01902	.02326
15		.01128	.01673	.02090
16	.00995	.00999	.01497	.01890
17		.00880	.01348	.01719
18	.00781	.00781	.01135	.01569
19		.00693	.01114	.01440
20	.00628	.00626	.01021	.01327
21		.00566		
22	.00516	.00512		
23		.00467		
24	.00431	.00427		
25		.00391		
26	.00365	.00360		
27		.003325		
28	.003134	.003078		
29		.002869		
30	.002717	.002663		
31		.002485		
32	.002378	.002325		
100	.000226			

Accurate formulæ were derived for calculating the percentage eliminated each generation in terms of the gametes produced by the preceding generation. The formulæ for describing the effects of this selection in terms of n the number of the filial generation are more or less crude approximations which may be useful for extending the curves somewhat beyond the point where they have been accurately derived.

When the type of selection complementary to the above takes place, that is, when all individuals in a polyhybrid stock, that show any recessive traits whatever, are eliminated, the effects can be stated much more simply, as the problem of these effects has been solved for all cases. The percentage of individuals to be eliminated each generation when plotted form a smooth curve, beginning with a value of $\frac{3\;m}{4\;m}$ in the F_2 generation and approximating zero at infinity (figure 1). As n becomes large the percentage eliminated each generation is in direct proportion to the number of factors involved (m) and in inverse proportion to the square of the number (n) of the filial generation F_n. These effects can be expressed as m/n^2. The type here eliminated disappears somewhat more rapidly than its complementary type.

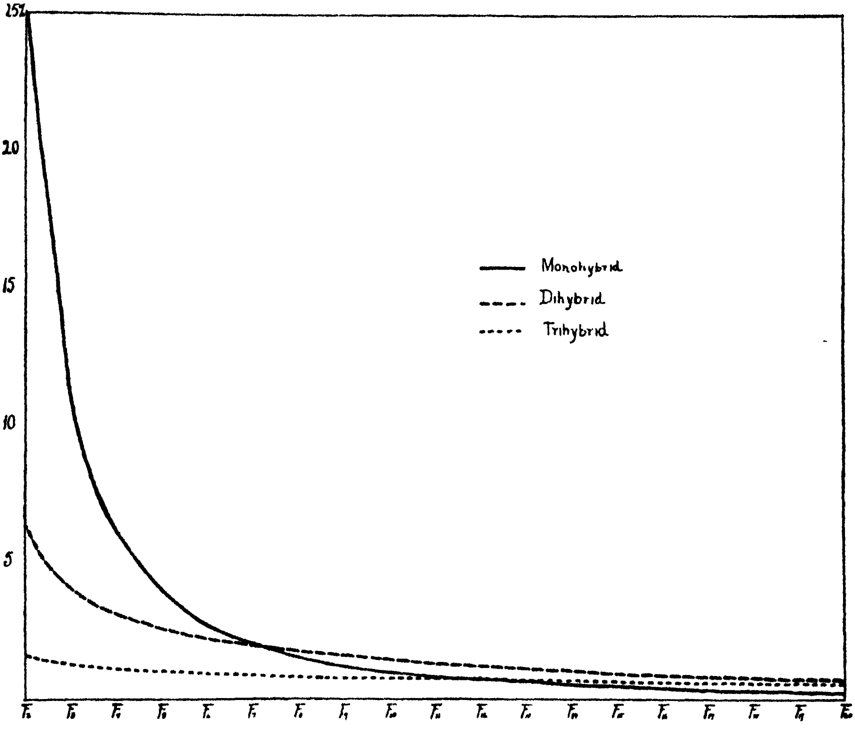

FIGURE 2.—Percentage of homozygous recessive individuals appearing each generation in a mono-, di-, and trihybrid stock when none such are allowed to breed and the rest of the stock bred at random.

When all individuals recessive for all m factors in a polyhybrid of m allelomorphs are eliminated the percentages of such individuals which appear in the various generations form, when plotted, curves of the same general shape as those just discussed. The percentage of homozygous recessives which appears in the F_2 generation is always $1/4^m$, thus as m

increases the curves start at much lower points (figure 2). The effects of the first selection (that which affects generation F_3) cause the completely recessive individual to disappear at the same rate regardless of the value of *m*. Thus the points F_2 and F_3 on the dihybrid curve occur on the monohybrid curve only further along (at F_4 and F_5) and the points F_2 and F_3 on the trihybrid curve occur at points still further along on the monohybrid (at F_8 and F_9). In the F_4 and subsequent generations of the polyhybrids the effects of "shifting" of gametes make themselves felt with the result that the eliminated type disappears much more slowly as *m* increases. Thus although in the F_2 generation the percentage of completely recessive individuals was 25 in the monohybrid and but 6.25 in the dihybrid, in generation F_7 they were approximately the same in the two (2.04 and 1.96 respectively), while in the F_{20} generation the percentage in the dihybrid was .66 to .25 in the monohybrid (table 36). Although the trihybrid curve has but a value of 1.56 in the F_2 generation it equals that of the monohybrid in generation F_{12} and subsequently to this its value decreases so much less rapidly than the mono- and dihybrids that at about generation F_{27} it crosses the dihybrid curve.

TABLE 36

Tables showing the percentage of completely recessive individuals, appearing each generation under conditions in which no such individual is allowed to breed.

GENERATION	MONOHYBRID ($1/n^2$)	DIHYBRID	$(n+2 \cdot 2.05)/n^2$	TRIHYBRID
F_2	.25000	.06250		.01562
F_3	.11111	.04000		.01234
F_4	.06250	.03095		.01093
F_5	.04000	.02564	.02380	.01006
F_6	.02777	.02222	.02161	.00939
F_7	.02040	.01957	.01938	.00883
F_8	.01562	.01744	.01737	.00833
F_9	.01234	.01566	.01564	.00789
F_{10}	.01000	.01417	.01414	.00748
F_{11}	.00826	.01288	.01286	.00711
F_{12}	.00694	.01180	.01175	.00677
F_{13}	.00591	.01082	.01078	
F_{14}	.00510	.00998	.00994	
F_{15}	.00444	.00925	.00921	
F_{16}	.00390	.00859	.00856	
F_{17}	.00346	.00801	.00799	
F_{18}	.00308	.00748	.00747	
F_{19}	.00277	.00702	.00701	
F_{20}	.00250	.00660	.00660	
F_{100}	.00010		.00080	.00216

A comparison of the results obtained by eliminating completely recessive individuals and by eliminating completely dominant individuals would indicate that, when the number of genes is the same in both cases, the recessive is eliminated much more slowly than the dominant. Thus in the F_2 generation of the dihybrid the percentage of individuals dominant for m factors is 56¼ while the percentage recessive for m factors is but 6¼, in generation F_{20} the percentage of completely dominant individuals has been reduced to .626 which is less than the percentage of complete recessives in this generation (.66). In generation F_{100} their values would be .023 and .081 respectively. In generation F_{100} of the trihybrid the complete recessive would also occur about four times as frequently as the complete dominant.

These results are in keeping with the findings of WARREN when he investigated the effects of selection upon a monohybrid stock under conditions approximating those found in nature. He found that the elimination of the less viable type was more rapid when it was of a dominant form.

When any combination of two or more dominants is eliminated from a polyhybrid of m allelomorphs in the F_2 and subsequent generations, it continues to appear in decreasing numbers. The greater the value of m, the greater the percentage of such combinations which appear in the F_2 generation and the more rapidly is the type eliminated (table 37).

TABLE 37

The percentage of individuals eliminated (all of those who have 2 or more dominant factors) in each generation from:

GENERATION	DIHYBRID	TRIHYBRID	TETRAHYBRID	OCTAHYBRID
F_2	.5625	.84375	.94922	.99952
F_3	.1632	.24000	.28402	.35840
F_4	.1189	.14958	.16064	.16856
F_5	.0876	.09683	.09729	.09150
F_6	.0658	.06557	.06355	.05700
F_7	.0505	.04698	.04428	.03866
F_8	.0397	.03504	.03242	.02786
F_9	.0309	.02700	.02467	.02100
F_{10}	.0260	02138	.01936	01637

Consequently when the percentage of the type is plotted against the filial generations, the curves of the various polyhybrids intersect as shown in figure 3.

Summary of formulæ

In the formulæ the symbols used have the following values: n = the number of the filial generation F_n, m = the number of genes concerned; c_1, c_2, c_3, c_4 · · · · c_{n+1} = the successive terms of the expanded binomial

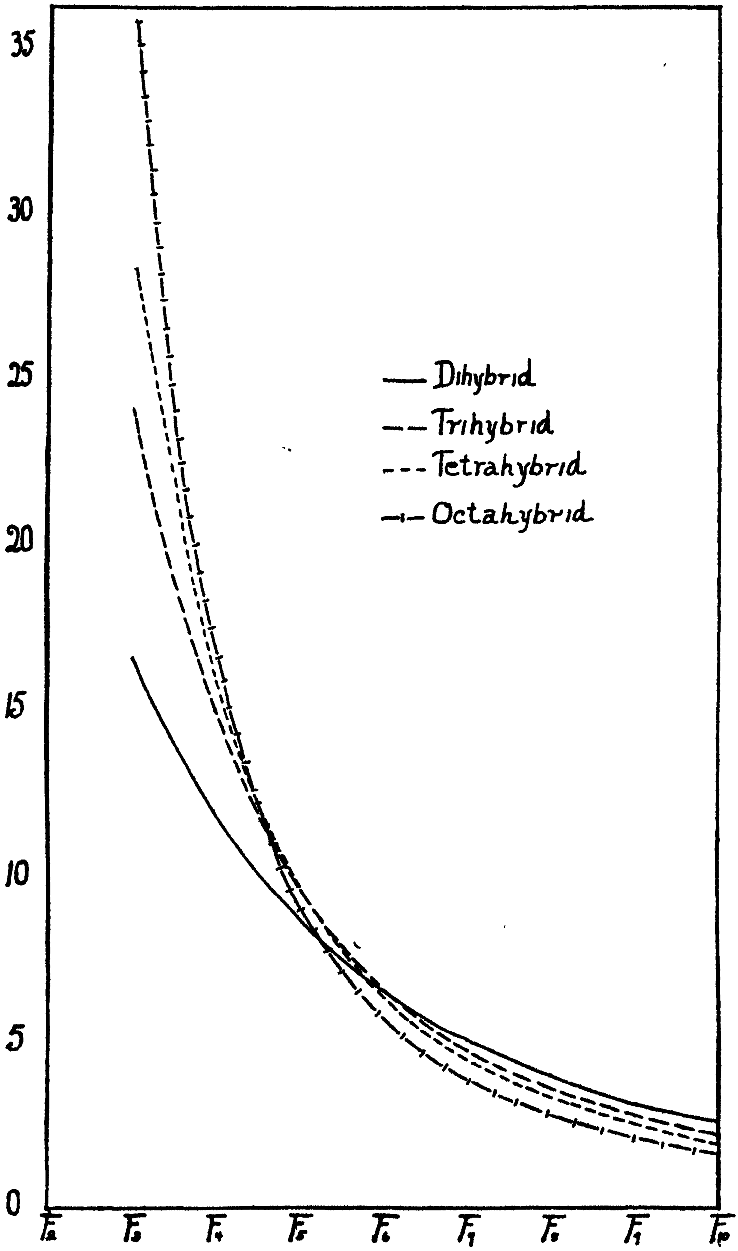

FIGURE 3.—Percentage of individuals containing two or more dominant factors in a di-, tri-, tetra- and octahybrid stock when none such are allowed to breed.

$(1+1)^m$; p = the group of gametes having all m dominant factors; q = the group having $m-1$ dominant factors; r = the group having $m-2$ and so on. In all instances the stock is completely heterozygous in the F_1 generation and selection starts in generation F_2.

I. Formulæ for calculating the percentage of completely dominant individuals appearing in a stock where none such are allowed to breed.

1. In the case of the dihybrid: Gametes of generation G_{n+1} in terms of gametes of G_n (In G_1 all gametes equal 1):

$$q_{n+1}=q_n(q_n+r_n)$$
$$r_{n+1}=r_n(2q_n+r_n)$$

The percentage of zygotes containing all m dominant factors in terms of any given set of gametes:

$$\frac{2q^2}{(2q+r)^2}$$

An approximate formula which gives very nearly the true values expressing the percentage in terms of n, when $n>7$:

$$\frac{2}{\left(n-\sqrt{\frac{n}{2}+1}\right)^2}$$

2. In the case of the trihybrid. The gametes are:

$$q_{n+1}=q_n^2+2q_nr_n+\frac{q_ns_n+r_n^2}{2}$$

$$r_{n+1}=2q_nr_n+r_n^2+2\frac{q_ns_n+r_n^2}{2}+r_ns_n$$

$$s_{n+1}=3\frac{q_ns_n+r_n^2}{2}+3r_ns_n+s_n^2$$

The percentage of zygotes containing all m factors in terms of the gametes:

$$\frac{6q(q+r)}{(3q+3r+s)^2}$$

A formula expressing the percentage in terms of n which is fairly accurate when $n>7$:

$$=\frac{2+\frac{\log(n-3)}{\log\ 4}}{n^2}$$

3. In the case of the tetrahybrid, the gametes are:

$$q_{n+1}=q_n^2+3q_nr_n+3\frac{q_ns_n+r_n^2}{2}+\frac{q_nt_n+3r_ns_n}{4}$$

$$r_{n+1}=2q_nr_n+4\frac{q_ns_n+r_n^2}{2}+r_n^2+2\frac{qnt_n+3r_ns_n}{4}+2_ns_n+\frac{r_nt_n+s_n^2}{2}$$

$$s_{n+1}=\frac{3q_ns_n+r_n^2}{2}+3\frac{q_nt_n+3r_ns_n}{4}+3r_ns_n+3\frac{r_nt_n+s_n^2}{2}+s_n^2+s_nt_n$$

$$t_{n+1}=4\frac{q_nt_n+3r_ns_n}{4}+6\frac{r_nt_n+s_n^2}{2}+4s_nt_n+t_n^2$$

The percentage of zygotes containing all m factors in terms of the gametes:

$$\frac{4q(3q+6r+2s)+6r^2}{(4q+6r+4s+t)^2}$$

A crudely approximate formula for expressing this percentage in terms of n, when $n>7$:

$$1+\frac{\log(n-3)}{\log\ 2}$$

$$n^{4}$$

Methods for deriving formulæ for other polyhybrids where $m>4$ are given in PART I.

II. Formulæ for calculating the percentage of individuals who show some successive character in a stock where only completely dominant individuals are allowed to breed.

1. In the case of the monohybrid:

$$\frac{1}{n^2}$$

2. In the case of the dihybrid:

$$\frac{2}{n^2}-\frac{1}{n^4}$$

3. In the case of the trihybrid:

$$\frac{3}{n^2}-\frac{3}{n^4}+\frac{1}{n^6}$$

4. In the case of the tetrahybrid:

$$\frac{4}{n^2}-\frac{6}{n^4}+\frac{4}{n^6}-\frac{1}{n^8}$$

5. In the case of the polyhybrid:

$$\frac{c_2}{n^2}-\frac{c_3}{n^4}+\frac{c_4}{n^6}-\frac{c_5}{n^8}\cdot\cdot\cdot\frac{1}{n^{2m}}$$

Expressed in terms of m:

$$\frac{m!}{1!m-1!n^2}-\frac{m!}{2!m-2!}+\frac{m!}{3!m-3!n^6}\cdot\cdot\cdot\pm\frac{m!}{m!0!n^{2m}}$$

Ignoring the latter term of the formula, whose value soon becomes negligible, and using only the first two terms:

$$\frac{m}{n^2}-\frac{m(m-1)}{2n^4}$$

As n increases the first term soon approximates the value of the entire formula:

$$\frac{m}{n^2}$$

III. Formulæ for calculating the percentage of completely recessive individuals in a stock where none such are allowed to breed.

1. For the monohybrid:

$$\frac{1}{n^2}$$

2. For the F_2 and F_3 generations of any polyhybrid:

$$\frac{1}{(n-2+2^m)^2}$$

3. In the case of the dihybrid the gametes are:

$$p_{n+1}=p_n{}^2+2p_nq_n+\frac{p_nr_n+q_n{}^2}{2}$$

$$q_{n+1}=p_nq_n+q_n{}^2+\frac{p_nr_n+q_n{}^2}{2}+q_nr_n$$

$$r_{n+1}=\frac{p_nr_n+q_n{}^2}{2}+2q_nr_n$$

The percentage of zygotes, homozygous recessive for all m factors:

$$\frac{r^2}{(p+2q+r)^2}$$

An approximate formula whose value is extremely close to the true value determined by the above when $n>7$:

$$\frac{\sqrt{n+2}-2.05}{n^2}$$

4. In the case of the trihybrid, the gametes are:

$$p_{n+1}=p_n^2+3p_nq_n+3\frac{p_nr_n+q_n^2}{2}+\frac{p_ns_n+3q_nr_n}{4}$$

$$q_{n+1}=p_nq_n+q_n^2+2\frac{p_nr_n+q_n^2}{4}+2q_nr_n+\frac{p_ns_n+3q_nr_n}{4}+\frac{q_ns_n+r_n^2}{2}$$

$$r_{n+1}=\frac{p_nr_n+q_n^2}{4}+2q_nr_n+\frac{p_ns_n+3q_nr_n}{2}+r_n^2+2\frac{q_ns_n+r_n^2}{}+r_ns_n$$

$$s_{n+1}=\frac{p_ns_n+3q_nr_n}{4}+3\frac{q_ns_n+r_n^2}{2}+3r_ns_n$$

The percentage of zygotes, homozygous recessive for all m factors:

$$\frac{s^2}{(p+3q+3r+s)^2}$$

A formula which crudely approximates the above:

$$\frac{4}{(\sqrt{8n-15}+15)^2}$$

Methods for deriving formulæ for other polyhybrids are given in PART III.

IV. 1. Formulæ for expressing the percentage of individuals containing 2 or more dominant factors in a polyhybrid of m allelomorphs when none such are allowed to breed.

The gametes are:

$$q_{n+1}=q_n(q_n+r_n)$$
$$r_{n+1}=r_n(mq_n+r_n)$$

The percentage of zygotes to be eliminated in terms of any given set of gametes is:

$$\frac{m(m-1)q^2}{(mq+r)^2}$$

2. Formula for expressing the percentage of individuals containing 3 or more dominant factors in a polyhybrid of *m* allelomorphs when none such are allowed to breed:

The gametes are:

$$q_{n+1}=q_n^2+2q_nr_n+\frac{q_ns_n+r_n^2}{2}$$

$$r_{n+1}=(c_2-1)q_nr_n+(c_2-1)\frac{q_ns_n+r_n^2}{2}+r_n^2+r_ns_n$$

$$s_{n+1}=c_3\frac{q_ns_n+r_n^2}{2}+c_2r_ns_n+s_n^2$$

The percentage of zygotes to be eliminated is:

$$\frac{q\left\{\left[(c_3c_3-1)q+2\left[c_2c_3-c_2+(c_{2-1})\right]r\right\}}{(c_3q+c_2r+s)^2}$$

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EFFECTS OF TEMPERATURE ON LENGTH OF VESTIGIAL WING IN *DROSOPHILA VIRILIS*[1]

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INTRODUCTION

While carrying on selection experiments with the vestigial wing form of *Drosophila ampelophila*, ROBERTS (1918) noted that during the summer months the length of the wing increased. He later placed some of the progeny in an incubator at about 28 degrees and the rest at room temperature of about 22 degrees. He concluded that the length of the vestigial wing increases with an increase in the temperature and that the males are affected more by high temperature than are the females.

The present paper records an attempt to ascertain whether low as well as high temperatures modify the wing length of Drosophila, and whether the change resembles that of a chemical reaction. It has been found in the case of homogeneous reactions that a rise of 10 degrees in the temperature increases the rate of reaction 2 to 4 times, or, on the average, by 2.5 times. This applies to all reactions, whether they take place under the influence of a catalyst or not. The rate of increase falls, to a greater or less extent, as the temperature rises. When one considers not single reactions but the complex life processes as a whole, it is found that in these cases also a similar relationship exists between temperature and rate of reaction so that a rise of 10 degrees in the temperature is accompanied by a doubling or a trebling of the rate at which life processes take place. This has been shown by WOODRUFF and BAITSELL (1911) with *Paramaecium aurelia* and LOEB and NORTHROP (1917) with Drosophila. They demonstrated that life is lived more rapidly at a temperature higher than normal.

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[1] I am indebted to Professor H. S. JENNINGS and Doctor ALEXANDER WEINSTEIN for suggestions in connection with this work.

MATERIAL AND METHODS

The offspring of the mating of a single pair of a vestigial stock of *Drosophila virilis* furnished by Doctor ALEXANDER WEINSTEIN were used as a basis of all the experiments. As a number of preliminary experiments were made during the winter of 1924–1925, these individuals were bred together for a whole year before the experiments recorded below were carried out. To insure virginity, the females were picked out of the breeding bottles within 3 hours after hatching. The matings were made with a single male and female in a breeding bottle. Individuals of nearly similar body size and wing length were mated in an attempt to make all conditions as nearly equal as possible. After they had been in the breeding bottle some time the pair were transferred to a second bottle and then to a third so as to secure eggs in three bottles. No attempt was made to count the number of eggs laid in each bottle, but the presence of eggs in each bottle was ascertained. One bottle was then placed at a low temperature (usually 12 degrees, maximum range 9 to 13 degrees), another at room temperature (18 to 22 degrees) and the third at a high temperature (29 to 30 degrees).

Many eggs placed at the low temperature failed to develop to maturity. It was difficult to determine whether this was due to a reduction in their vitality with a decrease in temperature or whether other factors in the environment such as bacterial changes in the banana agar, or mold, killed them. For the purpose of this comparative study only those families which produced progeny at all temperatures were used.

The offspring were preserved in 75 percent alcohol for three weeks before the measurements were made. The longitudinal vein III[2] of the right wing was measured in each case and this length used as a standard for comparison, and, in addition, the *tibia* of the right front leg was measured. In a few instances the distance between the eyes and the length of the thorax were also measured. These measurements were used as an index of the size of the individual. Measurements were made with the low power of a binocular microscope fitted with a measuring ocular in the right ocular. The ocular micrometer was calibrated against a Leitz stage micrometer.

The flies were fed in the usual manner with fermented banana, as described by MORGAN, STURTEVANT, MULLER and BRIDGES (1915). Some individuals were lost by sticking in the banana agar and a few had to be discarded owing to excessive curling of the wings, which prevented measuring.

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[2] The terminology is after MORGAN, BRIDGES and STURTEVANT, 1925, p. 6.

EXPERIMENTAL DATA

The results obtained may be illustrated by presenting a typical example (table 1) and a summary (table 2).

TABLE 1

Wing lengths of individuals of sub-family A12, in units each of which is one mu.

P	F_1		
	9°-13°	18°-22°	29°-30°
24.67	9.67	25.93	24.61
24.64	11.8	23.8	27.5
	12.75	23.55	19.9
	8.55	23.75	33.82
	9.5	18.64	28.73
	8.83	20.15	20.95
	12.91	19.1	39.44
	7.24	22.37	29.35
	5.28	18.75	
	13.37	17.74	
	8.4	18.8	
	10.5	20.57	
	7.89	20.35	
	10.57		
	10.32		

We may now compare the effect of temperatures on wing length with their effects on the velocity of chemical reactions. Using the well known formula for the temperature coefficient,

$$Q_{10}=\left(\frac{K_1}{K_0}\right)\frac{10}{T_1-T_0}$$

in which Q_{10} is the coefficient of the rate of increase in the reaction for a rise of 10 degrees C and K_1 and K_0 represent constants observed at the temperatures T_1 and T_0 respectively, it is found that the mean Q_{10} derived from the data presented in table 2 is 1.98 (see tables 3 and 4).

An analysis of tables 3 and 4 shows that the wing length of Drosophila is affected more by lowering the temperature below the usual condition than by raising the temperature. As already pointed out, this is to be expected on the basis of the effect on the speed of a chemical reaction. Although the Q_{10} in every case is somewhat lower than that obtained for a simple chemical reaction, the temperature coefficients are in striking agreement with the demands of the *van't* HOFF law.

The body size of the progeny of both small and large flies varied greatly and there appeared to be no particular correlation between the size of

TABLE 2

Average wing length of F_1 at different temperatures. (Unit equals one mu.)

CASE	F_1* WING LENGTH	9°–13°				18°–22°				29°–30°			
		NO. OF F_1	♂	♀	Both	NO. OF F_1	♂	♀	Both	NO. OF F_1	♂	♀	Both
A12	24.67 24.64	15	11.12	8.37	9.83	13	21.68	20.48	21.03	8	27.28	28.82	28.03
A40	20.20 19.96	9	9.69	12.78	11.29	20	17.51	18.20	17.99	6	35.10	30.16	30.98
C3	22.80 22.75	11	11.41	10.63	10.96	7	16.81	22.05	19.94	11	26.64	27.97	27.60
D25	20.36 20.00	11	8.55	8.00	8.25	13	19.15	21.21	20.43	12	29.82	31.64	30.41
D31	24.78 25.16	3	10.83	none	10.83	13	22.62	none	22.62	4	28.95	none	28.95
D32	17.45 17.30	5	11.12	14.20	11.73	14	18.13	22.53	20.50	4	36.19	28.00	30.05
D37	22 00 21.93	5	10.71	10.83	10 76	13	22.10	21.14	21.51	8	36.67	35.29	36.23
G2	23.50 23.60	4	10.69	11.36	10.70	5	21.35	20.02	20.82	8	33.68	31.38	32.53
H5	19.70 19.70	2	12.41	9.73	11.07	8	22.88	21.52	22.22	5	39.32	35.83	37.35
H20	19.38 19 43	7	9.63	12.79	11.44	6	21.55	23.37	22.46	9	37.05	33.95	35.67
H22	15.45 15.50	7	10.94	8.69	9.66	10	19.39	22.26	20.54	11	35.73	32.61	33.12
K7	18.56 18.62	2	12.25	none	12.25	16	21.97	21.19	20.36	8	31.02	29.14	29.61
Total average			10.77	10.73	10.73		20.42	21.36	20.86		33.12	31.34	31.71

* Reared at room temperature.

the body and the length of the wing. Some large flies had very short wings while many large individuals had long wings and *vice versa*. There was likewise no striking difference between the sexes.

The average length of time required for flies bred at 9 to 13 degrees to reach the Imago stage was 90 days; those kept at 18 to 22 degrees, 15 days; those raised at 29 to 30 degrees, 8 days.

TABLE 3

Temperature coefficient for temperatures below and above room temperature.

CASE	$Q_{10}(12°-20°)$	$Q_{10}(20°-30°)$
A 12	2.66	1.33
A 40	1.98	1.72
C 3	2.26	1.38
D 25	3.1	1.48
D 31	2.6	1.27
D 32	2.17	1.46
D 37	2.48	1.68
G 2	2.41	1.56
H 5	2.5	1.68
H 20	2.45	1.58
H 22	2.65	1.61
K 7	2.07	1 4

TABLE 4

Average temperature coefficient.

Average Q_{10} of all cases 12°–20°	2.44
Average Q_{10} of all cases 20°–30°	1.51
Average Q_{10} for all temperatures	1.98

The change in wing length due to temperature is not hereditary. F_2 from parents bred respectively at 12 degrees and at 30 degrees, when bred at 20 degrees had a wing length similar to those raised from parents at 20 degrees. In the case of F_1 raised at 30 degrees many individuals had apparently normal wings in respect to size and shape but were unable to fly.

SUMMARY

1. The vestigial wing length of *Drosophila virilis* is modified by changes in temperature in the manner of a chemical reaction. The temperature coefficient (Q_{10}) is approximately 1.98.
2. Low temperatures affect this character more than high temperatures. The temperature coefficient for the range between 12 and 20 degrees is approximately 2. 44, while that between 20 and 30 degrees is 1.15.
3. The changes in wing length due to temperature are not hereditary, when the offspring are bred at room temperature.
4. There is thus need of maintaining a constant temperature during the study of the genetics of wing length of Drosophila.

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CYTOLOGICAL AND GENETIC STUDIES ON FRAGARIA

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INTRODUCTION[1]

The eleven species and twelve cultivated varieties of the genus Fragaria which were included in this investigation may be divided into five different groups:

Group 1.—The European type, represented typically by the species *F. vesca* which is the most common wild species in Europe and is also widely distributed over NORTH AMERICA and western South America.

Fragaria vesca L.
F. americana Britton.

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[1] Several genetic investigations on the genus Fragaria were started at the BUSSEY INSTITUTION in 1921 with material furnished by Mr. G. M. DARROW of the UNITED STATES DEPARTMENT OF AGRICULTURE. Mr. DARROW has very kindly visited the laboratory several times, has identified and checked the living material, and has made many suggestions of great value during the progress of the work.

In 1924, Mr. K. ICHIJIMA undertook to make a cytological examination of all of the pure species and of the most interesting hybrids then on hand. This study he finished in the spring of 1925, but was unable to make a detailed report at that time because of a long illness. In the winter of 1925–1926, however, the present paper was written, and left to the undersigned to edit.

In the meantime, a paper entitled "Chromosomes and their significance in strawberry classification," by Dr. A. E. LONGLEY, was published in the Journal of Agricultural Research 32: 559–568, issued March 15, 1926. The data reported here corroborate many of Dr. LONGLEY'S results and furnish certain additional facts. Both papers gain in value from having been written simultaneously and independently.

E. M. EAST

F. californica Cham. & Schlecht.
F. mexicana Schlecht.
F. Helleri Holz.
F. bracteata Heller.

Group 2.—The Haut-bois type, represented by *F. elatior*, indigenous to Europe.

Fragaria elatior Ehrh.

Group 3.—The American type, represented typically by the species *F. virginiana* which is very widely distributed in North America.

Fragaria virginiana Duchesne.
F. glauca Rydb.

Group 4.—The Chilean type, indigenous to the Pacific Coast of North and South America and represented by the species *F. chiloensis*.

Fragaria chiloensis Duchesne.
F. cuneifolia Nutt? from Oregon.

Group 5.—Certain cultivated varieties of a type often described as *F. grandiflora* in the Taxonomy of the genus.

Varieties used:

William Belt
Champion Early
New York
La Pearl
Clark's Seedling
Doctor Burrell
Chesapeake
Ettersburg
Gardners
Progressive
Success

In addition to the above, the following hybrids were examined:

F_1 hybrids between the species—

F. vesca ♀ ×*F. Helleri* ♂
F. vesca ♀ ×*F. americana* ♂
F. Helleri ♀ ×*F. americana* ♂
F. bracteata ♀ ×*F. Helleri* ♂ . . . . (type a)
F. bracteata ♀ ×*F. Helleri* ♂ . . . . (type b)
F. glauca ♀ ×*F. virginiana* ♂
F. bracteata ♀ ×*F. virginiana* ♂
F. mexicana ♀ ×*F. virginiana* ♂
F. bracteata ♀ ×*F. glauca* ♂
F. americana ♀ ×*F. glauca* ♂
F. grandiflora var. *Dunlap* ♀ ×*F. platypetala* ♂

RYDBERG'S (1912) descriptions have been followed in making up the above list. The species which he designates as *F. Helleri*, however, is merely a pink-flowered variety of *F. vesca*.

METHODS

Maturation divisions occur very early in Fragaria flowers, and the chromosomes are very minute. For these reasons it was necessary to modify the cytological methods ordinarily in use in several particulars.

Flower buds were collected when about 1 mm in diameter, the calyx stripped off and fixed for 12 hours. For somatic chromosome counts, very young, thick root-tips from newly produced runners were found to be the most suitable material. As fixatives, Flemming's medium solution and chrom-acetic acid were found to be unsatisfactory. Bouin's solution as modified by ALLEN (1916) gave much better results, except in the hybrid between *F. bracteata* and *F. Helleri*, where straight Bouin's solution was used.

The material was washed 5 hours in water and then moved up through the alcohols. It was found necessary to transfer slowly to xylol, keeping the material 4 hours each in various percentages beginning with 15 percent.

The paraffine blocks cut very satisfactorily and the material examined unstained was not shrunken. But if care was not taken in staining, shrinkage was quite marked.

The best method of staining was found to be the use of 4 percent iron-alum as a mordant for only 2 hours, followed by transfer to haemotoxylin for 3 hours. No destaining was necessary.

CHROMOSOME DETAILS

a. *Chromosomes in Group 1—the European type*

All of the six species of this group which were investigated have 7 pairs of chromosomes in the pollen mother cells. No irregular behavior of the chromosomes in the course of the heterotypic division was observed. In the earliest stage the pollen mother cells are closely packed in the anther cavity. As the meiotic division proceeds the cells gradually begin to separate from one another. After the synapsis there is generally a distinct spireme stage in which the double thread-like nature can clearly be seen; but no second contraction has been observed. At early diakinesis the paired chromosomes are often unequal in size (figure 1), but gradually contract into a uniform size in the later stage. At this stage the 7 pairs of chromosomes can be seen clearly (figure 2). When the paired chromosomes become almost spherical the nucleolus and nuclear membrane disappear. The chromosomes then gather toward the center and arrange themselves on the equatorial plate. It is here at the metaphase and at the late anaphase that the chromosomes can be most readily counted (figures

3, 4). After the telophase and the subsequent resting stage the cells begin the homeotypic division which is carried out in the regular manner (figure 5), the resulting tetrads being quite normal. The 14 somatic chromosomes are seen clearly and may be counted readily at the metaphase of the root-tip cell (figure 6). The somatic chromosomes appear in a characteristic figure and are all separated from one another in well fixed preparations.

b. *Chromosomes in Group 2—The Haut-bois type*

In the single species, *F. elatior,* belonging to this group, the gametic number of chromosomes in the pollen mother cell is 21. Figure 7 shows the chromosomes in the heterotypic metaphase. There is no irregular behavior of the chromosomes during the whole process of maturation which indicates the stability of the species cytologically.

c. *Chromosomes in Group 3—The American type*

Two species of this type were investigated, *F. virginiana* and *F. glauca.* Both of them possess 28 chromosomes as the gametic number. Owing to the minute size of the pollen mother cell it is quite difficult to count the chromosomes when such large numbers are present. After a number of experiments it was found that counts could best be made in the late diakinesis of the heterotypic division (figure 8). The somatic chromosomes are closely and irregularly packed in the small root-tip cells. The somatic number of chromosomes could not be determined definitely although it appears to be approximately 56.

d. *Chromosomes in Group 4—The Chilean type*

F. chiloensis and *F. cuneifolia* both show 28 chromosomes as the gametic number. The behavior of the chromosomes is quite regular throughout both the heterotypic and the homeotypic divisions. No abnormalities in chromosomal behavior were found (figures 9, 10).

e. *Chromosomes in Group 5—The Cultivated variety type*

Twenty-eight pairs of chromosomes were in found the pollen mother cells of each of these varieties. Abundant pollen grains are produced and the process of reduction appears to be regular. There is little difference in the chromosome behavior between this type and the American type, both of which have the same number of the chromosomes (figure 11).

PLATE I

FIGURE 1.—The 7 paired chromosomes at diakinesis in *Fragaria vesca* var.

FIGURE 2.—A later stage of the same.

FIGURE 3.—Metaphase of heterotypic division.

FIGURE 4.—Late anaphase.

FIGURE 5.—Metaphase of homeotypic division.

FIGURE 6.—Metaphase of division in the root-tip cell (14 somatic chromosomes).

FIGURE 7.—Metaphase showing 21 pairs of chromosomes (*F. elatior*).

FIGURE 8.—Diakinesis showing 28 paired chromosomes (*F. virginiana*).

FIGURE 9.—The same stage in *F. chiloensis*.

FIGURE 10.—Homeotypic metaphase of the same.

FIGURE 11.—Diakinesis showing 28 paired chromosomes (a cultivated variety).

FIGURE 12.—Metaphase of the F_1 hybrid of *F. bracteata*×*F. Helleri*, showing two different groups of chromosomes.

FIGURE 13.—Diakinesis of the same (14 paired chromosomes).

FIGURE 14.—Homeotypic metaphase of the same (14 chromosomes).

FIGURE 15.—Root-tip cell of the F_2 plant of *F. bracteata*×*F. Helleri*, showing 28 somatic chromosomes.

FIGURE 16.—Diakinesis of reduction division of F_1 of *F. bracteata*×*F. virginiana* showing 7 bivalents and approximately 21 univalents.

FIGURE 17.—Metaphase of the same.

FIGURE 18.—Early metaphase of the same,—univalents lagging behind.

FIGURE 19.—Late anaphase of the same.

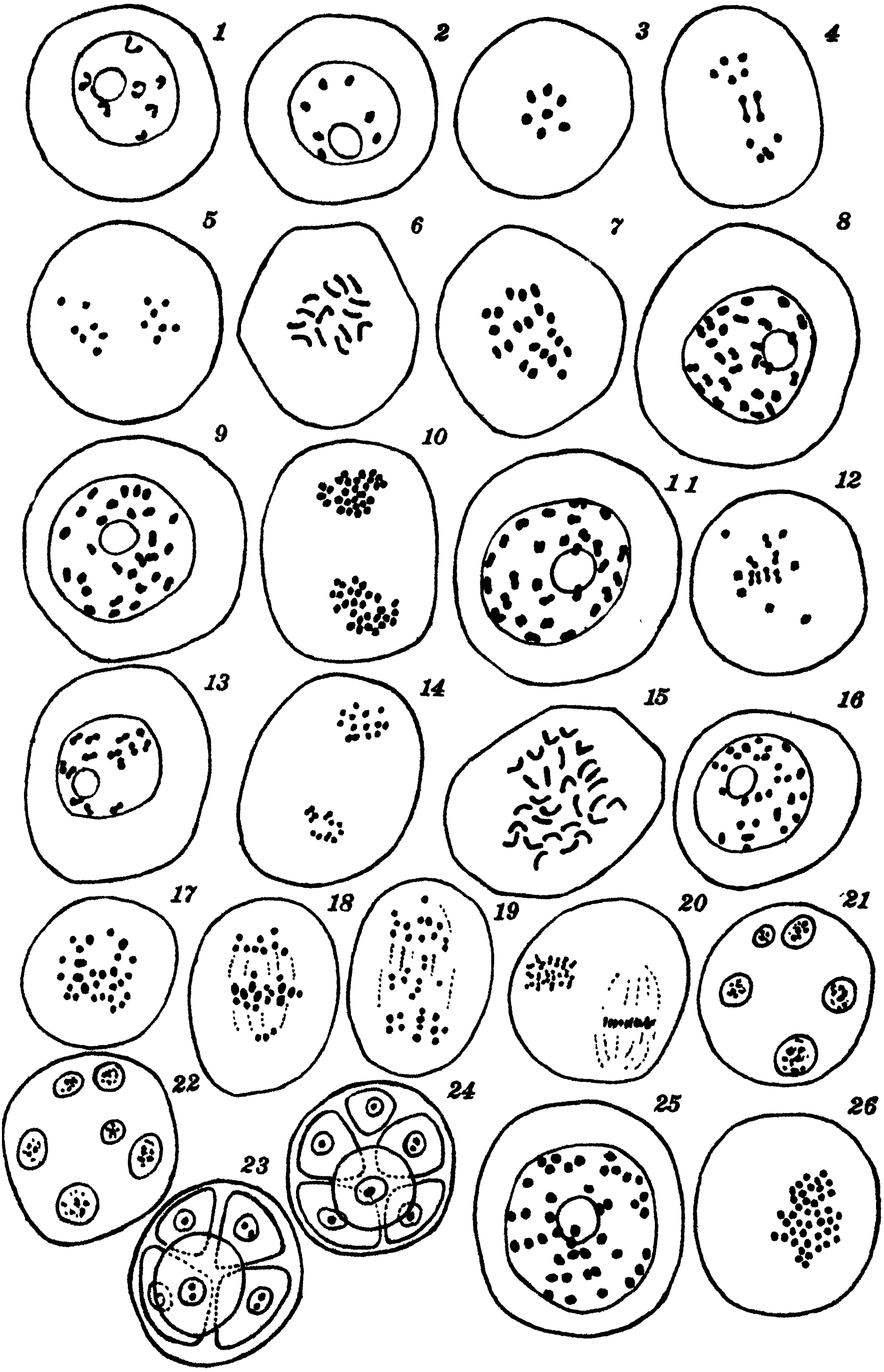
FIGURE 20.—Homeotypic metaphase of the same.

FIGURES 21–24.—Abnormal tetrad formations of the F_1 of *F. bracteata*×*F. virginiana*.

FIGURE 25.—Diakinesis in *Duchesnea indica*.

FIGURE 26.—Metaphase of the same.

Plate I



f. *Chromosomes in F_1 hybrids*

F_1 hybrids between species of the European type, such as *F. vesca*×*F. Helleri*, *F. vesca*×*F. americana*, *F. Helleri*×*F. americana*, and *F. bracteata* ×*F. Helleri* possess 7 pairs of chromosomes. Ordinarily there is no irregular behavior of the chromosomes, but an interesting exception has been found in the F_1 of one such cross. An individual in one of the populations of F_1 hybrids between *F. bracteata*×*F. Helleri* was found showing 14 pairs of chromosomes (figure 13) in the diakinesis of the pollen mother cell. Presumably the 7 chromosomes contributed by the bracteata parent and the 7 chromosomes contributed by the *Helleri* parent had doubled during some abortive attempt at cell division. Figure 12 shows one group of 7 chromosome pairs in the heterotypic metaphase which is easily distinguishable from a second group of 7 chromosome pairs. In the homeotypic division 14 chromosomes as a haploid number may be counted clearly (figure 14). The behavior of the gametic chromosomes seems to be normal. The F_2 hybrids obtained by selfing this plant showed 28 chromosomes in root-tip preparations (figure 15). The writer has not been able to investigate conditions in the pollen mother cells of the F_2 plants.

In F_1 hybrids between the species of the American type, for example *F. glauca*×*F. virginiana*, no abnormal chromosome behavior was observed. But hybrids between species of the different types, for example American type $(x=28)\times$European type $(x=7)$, which can be obtained only with difficulty, show many irregularities. Among a population of F_1 hybrids between *F. bracteata*×*F. virginiana* some individuals were found having 28 chromosomes in the heterotypic metaphase, of which 7, presumably bivalents, appear to be larger than the other 21 (figure 17). In the early metaphase there are approximately 28 chromosomes of which 7 bivalents are on the equatorial plate, while 21 univalents are found on the spindle (figure 18). Figure 19 shows 7 bivalents which, having divided, are pushing toward the poles, while the 21 univalents are lagging in their approach to the poles. It is obvious that each of the bivalents divides before moving to the poles, but the univalents pass at random to either pole without dividing. Owing to the minute size of the chromosomes, it is quite difficult to count the number of the bivalents and univalents in the heterotypic division. In figures 21–24 one may observe clearly the irregularity of the tetrad formation, a consequence to be expected from the previous behavior of the chromosomes. This irregularity of tetrad formation supplies the cytological basis of the practically complete sterility of these hybrids.

DISCUSSION

a. *Polyploidy in Fragaria species*

The foregoing investigation gives us another case of possible polyploidy in plant species. If the basic haploid chromosome number is taken to be 7, various Fragaria species may be interpreted as diploid, tetraploid, hexaploid and octaploid forms, that is, as forms having 7, 14, 21 and 28 pairs of chromosomes. Furthermore, *Duchesnea indica*, which once was classified as a species of Fragaria is found to have 42 pairs of chromosomes; and since it may be crossed with *Fragaria vesca*, it may possibly be regarded as a dodecaploid Fragaria (figures 25, 26).

The occurrence of chromosomes in a progressive arithmetical series has been found in several other genera. In Chrysanthemum TAHARA (1915, 1921) found species with haploid chromosome numbers 9, 18, 27, 36 and 45. In Viola, according to MIYAJI (1913) and MARCHAL (1920), there are species with 6, 10, 12, 24 and 36 haploid chromosomes. In Hieracium ROSENBERG (1917) found somatic numbers 18, 27, 36 and 54. In Crepis there are species with 6, 8, 10, 16, 18, 24 and 42 somatic chromosomes (ROSENBERG 1918, 1920). In Triticum SAKAMURA (1918, 1920) and SAX (1921, 1922) state that one species has 7 chromosome pairs, four species have 14 pairs, and three species have 21 pairs. In Campanula MARCHAL (1920) found 17, 34 and 51 haploid numbers. In Rubus LONGLEY (1924) found 7, 14 and 21 chromosome pairs. TÄCKHOLM (1920, 1922) and BLACKBURN and HARRISON (1921), investigating the genus Rosa, have established the most remarkable series with forms having 14, 21, 28, 35, 42 and 56 somatic chromosomes.

The European type of Fragaria which has the haploid chromosome number 7 may perhaps be regarded as the most primitive species of the genus. This species not only possesses the lowest chromosome number, but also its morphological characters appear to be the most primitive when compared with those of *F. elatior*, *F. virginiana*, and *F. chiloensis* which more closely resemble the horticultural varieties having higher chromosome numbers and more complex morphological characters. Furthermore the species of the European type are hermaphroditic, while certain species of the American type and of the Chilean type are partially diœcious.

No wild species were found possessing 14 pairs of chromosomes. Evidence of tetraploidy in Fragaria, therefore, rests upon a single case obtained in the cross between *F. bracteata* ($x=7$) and *F. Helleri* ($x=7$). The ordinary F_1 plants obtained from this cross were intermediate

PLATE II

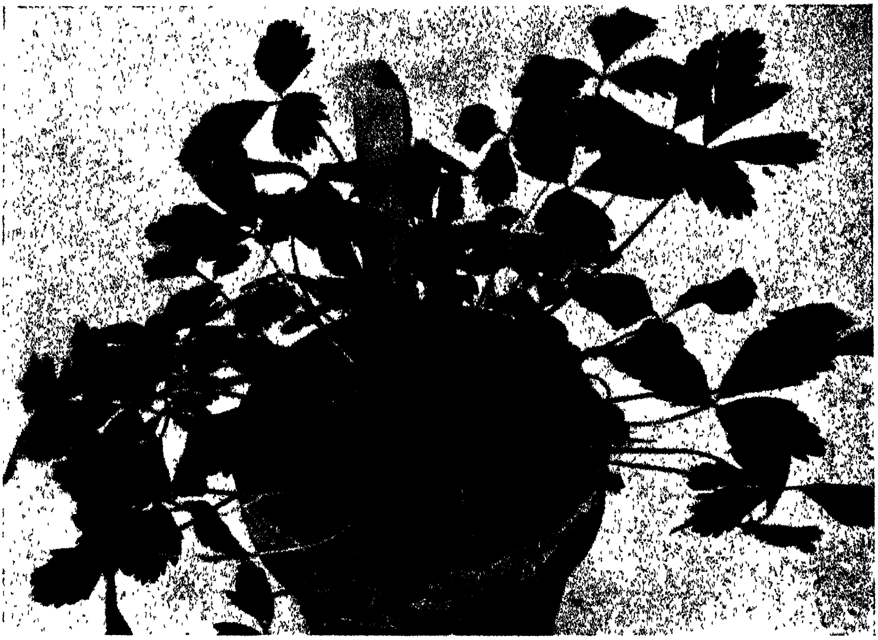


FIGURE A.—*F. bracteata*. A species of the European type.

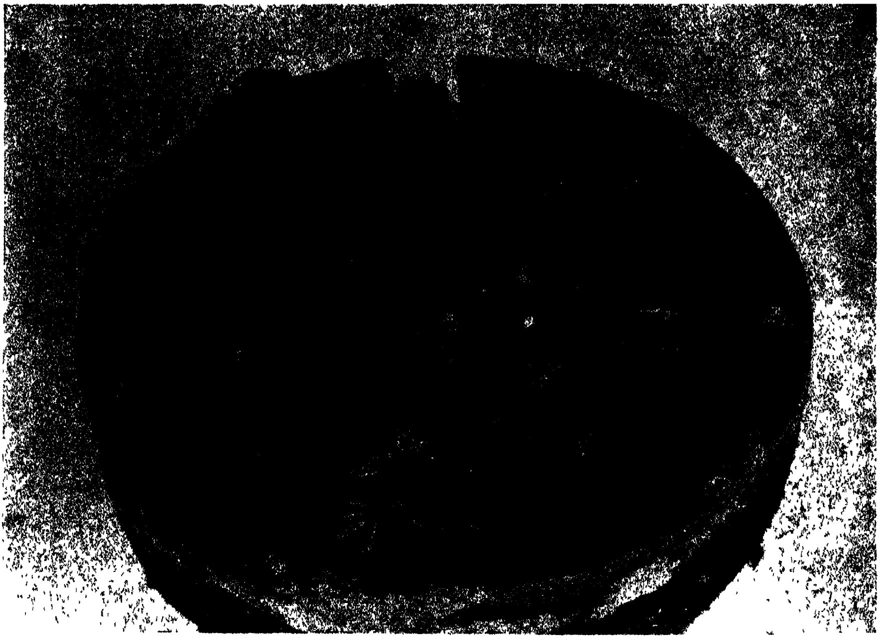


FIGURE B.—Hybrid, type b, of *F. bracteata*×*F. Helleri*.

PLATE III

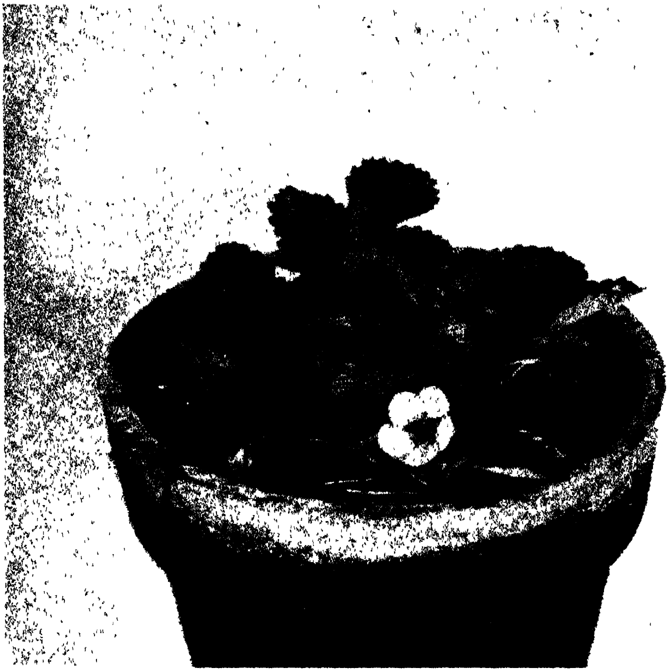


FIGURE A.—*F. virginiana*, a typical American type.

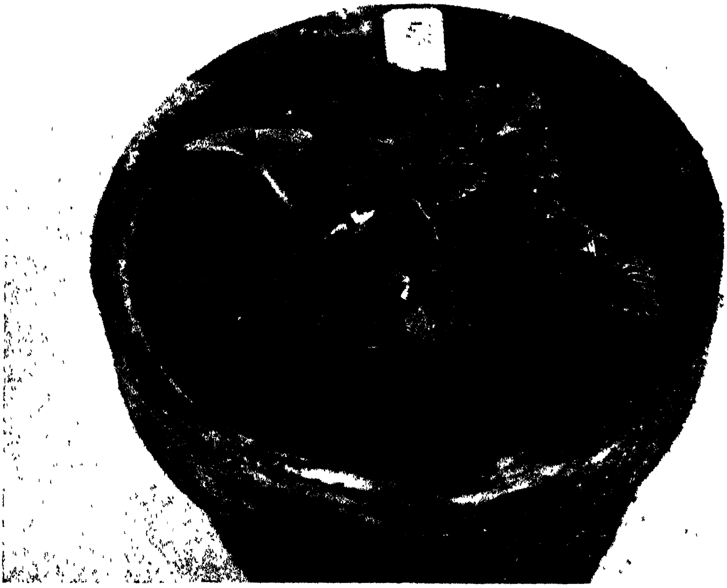


FIGURE B.—Hybrid of *F. bracteata*×*F. virginiana*.

between the parents except for flower color, the pink flower characteristic of *F. Helleri* being dominant. Nine of these ordinary hybrids (type a) were investigated cytologically and were found to have 7 pairs of chromosomes. The behavior at reduction was normal. The exceptional individual had 14 pairs of chromosomes, and instead of segregating and recombining the characteristics of the parental species as did the ordinary F_2 populations, it produced a uniform population of a new type. The leaves were larger, thicker and more crenate than either of the parents. The petals also were thicker and the pollen grains were decidedly larger than in the forms from which it arose (Plate 2, A and B). The root-tip cells of seven individuals were examined, and in each case 28 chromosomes were found. These plants may therefore be considered to be representatives of a new species.

Several other cases of plant tetraploidy which occurred under observation have been reported. *Oenothera gigas*, a mutant form of *O. Lamarckiana*, is a well known case (LUTZ 1907). In Primula DIGBY (1912) found a tetraploid form with 36 somatic chromosomes, and in Datura a tetraploid form has been found which has 48 somatic chromosomes instead of the 24 of the original species (BLAKESLEE, BELLING and FARNHAM 1920). In Nicotiana, CLAUSEN and GOODSPEED (1925) produced a true-breeding hybrid between glutinosa ($x=12$) and tabacum ($x=24$) which possessed 36 pairs of chromosomes.

b. *Species hybrids*

In the F_1 hybrids of *F. bracteata* ($x=7$)$\times$*F. virginiana* ($x=28$), as previously stated, there are 7 bivalents and 21 univalents in the heterotypic spindle. In the reduction division the bivalents divide normally while the univalents pass at random, without dividing, to either pole. The behavior of these chromosomes in the second division has not been clearly observed. But the subsequent tetrad formation seems to be much disturbed, because the majority of the pollen mother cells form three, five or six daughter nuclei instead of the normal four, none of which are able to become normal mature pollen grains. The irregularities of the chromosome behavior show the cytological basis of the sterility of the hybrid. This view has been borne out in several other species hybrids (See Plate 3, A and B).

Similar chromosomal irregularities in the heterotypic division of hybrids have been investigated in many other species. In Drosera ROSENBERG (1909) has investigated a hybrid between a species with 10 haploid and a species with 20 haploid chromosomes which shows

10 bivalent and 10 univalent chromosomes at diakinesis. GATES (1909) found the chromosome number of the F_1 of certain Oenothera crosses to be 20 or 21, the sum of the gametic number of the parents. In an F_1 hybrid of *Hieracium auricula* (x = 9) × *H. aurantiacum* (x = 18), 9 bivalents and 8 or 9 univalents were found in the reduction division (ROSENBERG 1917). In Triticum KIHARA (1919) described 14 bivalents and 7 univalents in the heterotypic prophase of the F_1 hybrid *T. polonicum* (x = 14) × *T. spelta* (x = 21). YASUI (1921) found a similar chromosome behavior in Papaver hybrids. TÄCKHOLM (1920, 1922) found that species of the Canina section in Rosa have usually 7 bivalents with 14, 21 or 28 univalents in the reduction division. SAX (1922) reports that in the F_1 of *Triticum monococcum* (x = 7) × *T. turgidum* (x = 14), there are 7 bivalents and 7 univalents in the heterotypic division; and the F_1 of the Emmer group (x = 14) × the Vulgare group (x = 21) shows 14 bivalents with 7 univalents in the first meiotic division, resulting in microspores having from 14 to 21 chromosomes.

SUMMARY

1. The Fragaria species investigated may be divided according to their chromosome number into four groups having 7, 14, 21, and 28 chromosomes respectively.
2. F_1 hybrids between different representatives of the American type have 28 gametic chromosomes.
3. F_1 hybrids between different representatives of the European type have 7 gametic chromosomes, with the exception of the following case.
4. The cross of *F. bracteata* × *F. Helleri* produced, in one case, a tetraploid form, having 14 gametic chromosomes, of which 7 are contributed by one parent and 7 by the other parent. The F_2 plants which were obtained by selfing this tetraploid form have 28 somatic chromosomes in the root-tip cells, and possess distinct morphological characteristics which have not been observed in any other species. The type may be regarded as a new species.
5. In the F_1 hybrid of *F. bracteata* × *F. virginiana* 7 bivalents and approximately 21 univalents were found. The bivalents divide normally, but the univalents pass at random to either pole without dividing. The tetrad formation seems to be greatly disturbed, consequently there are no normal mature pollen grains found.
6. *Duchesnea indica* is found to have 42 gametic chromosomes; and judging from possibility of crossing with *F. vesca*, it may possibly be considered to be a dodecaploid form of Fragaria.

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All drawings were made with the aid of the camera lucida. All figures were drawn from single sections. The lenses used were a 1.5-mm. Zeiss apochromatic objective and a No. 10 compensating ocular.

FURTHER EVIDENCE ON THE FACTOR FOR BITTERNESS IN THE SWEET ALMOND

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In the July 1923 issue of GENETICS in an article entitled "The Factor for Bitterness in the Sweet Almond," the writer presented data from an almond breeding experiment started in 1916 which seemed to show that as far as their genetic constitution for bitterness and sweetness is concerned, all varieties of sweet almonds are hybrids. This assumption was based on the fact that out of 243 seedlings derived by crossing different varieties of sweet almonds which came into bearing in 1922, 184 possessed sweet nuts and 59 possessed bitter nuts, or in terms of four in the ratio of 3.028 : 0.972, a very close approximation to the theoretical Mendelian monohybrid ratio of 3 : 1.

Since the report referred to above was first made, 480 additional trees have come into bearing so that at the present time data are available from a total of 723 trees. The following table shows the crosses made and the number of trees bearing sweet or bitter nuts in each cross:

Cross			Sweet trees	Bitter trees
Lewelling	×	IXL	21	4
California Paper Shell	×	Nonpareil	20	5
Ne Plus Ultra	×	Drake	2	0
Ne Plus Ultra	×	IXL	34	10
Nonpareil	×	Peerless	9	4
California Paper Shell	×	IXL	4	5
Lewelling	×	Ne Plus Ultra	5	3
California Paper Shell	×	Ne Plus Ultra	2	1
Reams	×	Ne Plus Ultra	5	1
Nonpareil	×	California Paper Shell	27	6
Reams	×	California Paper Shell	8	0
Nonpareil	×	Texas	21	4
Nonpareil	×	Ne Plus Ultra	13	8
Drake	×	Jordan	7	0
Drake	×	Ne Plus Ultra	43	17
Reams	×	Nonpareil	4	3
Reams	×	IXL	2	2
Languedoc	×	Drake	14	7
Reams	×	Texas	5	3
Ne Plus Ultra	×	Lewelling	29	6
Drake	×	IXL	23	5
California Paper Shell	×	Jordan	9	0

Ne Plus Ultra	×	Harriott	12	0
Lewelling	×	Nonpareil	4	0
Nonpareil	×	IXL	17	7
I×L	×	Ne Plus Ultra	7	4
King	×	Ne Plus Ultra	10	0
Drake	×	Languedoc	9	1
Nonpareil	×	Drake	13	12
Ne Plus Ultra	×	Nonpareil	17	7
Drake	×	Peerless	19	14
Languedoc	×	Ne Plus Ultra	2	1
Peerless	×	California Paper Shell	1	0
Golden State	×	Sellers	2	0
Drake	×	Texas	25	4
Drake	×	California Paper Shell	51	13
Texas	×	Drake	2	2
California Paper Shell	×	Drake	15	7
Harriott	×	Big White Flat	10	0
Nonpareil	×	Eureka	4	0
Drake	×	Eureka	2	0
Sellers	×	Drake	3	1
Lewelling	×	California Paper Shell	5	3
Ne Plus Ultra	×	California Paper Shell	3	2
I×L	×	Reams	2	1
Languedoc	×	IXL	1	0
		Total	547	176

An examination of the above table shows that there is no uniform outstanding proportion between trees possessing sweet almonds and those possessing bitter almonds in all the crosses. However, when the totals are examined, it will be noticed that there is nearly a perfect 3 : 1 ratio, the actual ratio being 3.108 : 1. It will also be observed that in only one case are there more bitters than sweets; namely, in the California Paper Shell×IXL cross in which 5 trees out of 9 possess bitter nuts. It will also be seen that in some of the crosses no bitter nut bearing trees have appeared.

With the larger population of trees available for study the assumption made in the first report that all sweet almonds are probably heterozygous for sweetness as the dominant character and the factor for bitterness as the recessive seems to be substantiated with the possible exceptions of the case mentioned above and the two crosses having the Harriott as one of the parents, in one case Na Plus Ultra×Harriott and the other Harriott ×Big White Flat in neither of which any bitter nut trees appeared. The total of twenty-two individuals in these two crosses, all of which possess sweet nuts is a fairly good indication that Harriott is possibly homozygous for sweetness.

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